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Effect of *Neem* extract on multidrug resistant *Escherichia coli* isolated from broiler chickens

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Abstract: The goal of this work was to focus on the efficacy of aqueous extract of 10% *Neem leaves* against colibacillosis, two hundred broiler chickens samples were collected from different localities of El-Sharkia governorate, Egypt, (52%) were positive for *E.coli*. The highest incidence was O_6 (23%), while O_{119}, O_2 and O_{146} were (15.4%). Disk diffusion method showed variable range of antibiotic resistance which revealed high prevalence of multidrug resistance phenotypes among isolates. Studying genotypic attributes of isolates using PCR, all tested E.*coli* isolates were positive for virulence genes (*pho*A and *iss*) (100%),while *(iroN* and *omp*T) (80%), in addition, *bla*CTX-M antibiotic resistance gene was shown positive 100% in all MDR *E.coli*, while (*aad*AI and *qnr*S) (90%),(*erm*B and *tet*A) (80%) and (*sul*I) (70%). The isolated APEC O_{119} 0.5 ml (1.0×10^8 CFU) used to induce an organized experimental study on eighty, one day old chicks, the clinical sings and the postmortem lesions were recorded, also histopathological, hematological and biochemical examination were be performed. The hematological results revealed macrocytic normochromic anemia, leukocytosis, heterophilia, monocytosis and lymphopenia in infected group , biochemical results revealed the increase alteration in liver and kidney function tests, antioxidant, anti-inflammatory marker, immunoglobulins IgA and IgM, while prophylaxis in group E ameliorate that occurred in all examined parameters. The study suggest that using 10% *Neem* leaves extract as a prophylaxis in addition to amoxicillin ameliorate the alteration in hematological , biochemical , inflammatory markers , antioxidant, immunoglobulin's and histopathological changes of all examined organ in infected broilers by *E.coli.*

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Keywords: Colibacillosis - Antibacterial – Broiler chicken- Hematology – *Neem* extract- multidrug Resistant *E.coli* –Virulence gene- Resistant gene- Histopathology.

1. Introduction

Colibacillosis caused by Avian pathogenic *E. coli* (APEC) is the most serious diseases affecting poultry production in Egypt which is an important cause of economic loss in poultry industry due to high mortality and decreased feed conversion **(Barnes *et al.*, 2008)**, also there is increasing evidence suggesting that APEC is a zoonotic pathogen **(Rodriguez *et al.*,2005).**The global incidence of food born infections has markedly increased especially in developing countries with poor hygiene and sanitation. *Eschetrichia coli* is the major causes of outbreaks of food borne diseases with significant mortality rate among the young and elderly **(Ahmed and Shimamoto, 2014).** *Eschetrichia coli* is one of the most important etiologic agents of enteritis, respiratory disease, septicemia, swollen head syndrome **(Barnes *et al.*, 2008).**

Antibiotics act as inhibition and killing the growth of bacteria, so it was used for controlling bacterial infections. Indiscriminate and long use of antibiotics leads to increase antibiotic resistance in bacteria, also decrease the number of beneficial gut microbiota in poultry **(Diarra and Malouin, 2014)**. Excessive use of antibiotics results residues in

poultry meat which affect consumer's health **(Mehdi et al.,2018).**

Hence, rapid identification of pathogenic *E. coli* strains and molecular detection of their resistance and virulence genes allow rapid diagnosis of pathogenic *E. coli* strains and understand their genetic and pathogenic relatedness, in order to improve diagnostic protocol **(Oswald *et al.*,2000 and Tetsuo *et al.*,2011).**

Increasing failures in antibiotic therapy which exhibited by microbial pathogens has screened of several medicinal plants for their potential antimicrobial activity **(Scazzocchio *et al.*,2001).** Recently, several efforts have been directed to control infectious diseases by use of herbal medicine which have ecologically safe and fewer side effects. *Neem* (*Azadirachta indica*) is one of well-known for its medicinal properties **(Nayaka *et al.*,2013)**, belongs to family Meliaceae which is evergreen tree of potential medicinal value **(Wafaa *et al.*,2007)**. *Neem* is the most useful traditional medicine which act as source of many therapeutic agents in the tropical and semi-tropical countries **(Landy *et al.*,2011)**, also it acts as a growth promoter improve performance, hematological parameters and immune response in broilers **(Jawad *et al.*,2013)**. The industrial and medicinal uses of *Neem* tree have been reviewed **(Schmutter , 1995).**

Therefore, the present study was undertaken to study the biochemical, immunological, antioxidants, antibacterial and pathological effects of experimentally infected multidrug resistant APEC on 1-day-old chicks supplemented with *Neem* leaves extract with focusing a highlight on virulence and resistant genes of *E.coli* isolates by PCR.

2. Materials and Methods

2.1. Samples' collection.

Two hundred samples were collected from different localities of Sharkia province in Egypt, diseased and freshly dead broiler chickens (4 to 6 weeks) which suffer from depression, off food, ruffled feather, dropping of wings and diarrhea. Specimens of liver, heart, lung and spleen of diseased chickens were collected aseptically in sterile separate plastic bags, pooled, labeled and submitted in ice box to the Reference Laboratory of Veterinary Quality Control of Poultry Production (RLQP) from February to July 2021.

2.2. Bacterial isolation and identification.

2.2.1. Bacterial examination

Isolation of *E. coli* was carried out according to **(Lee *et al.*,2008)**, *E. coli* suspected colonies were confirmed biochemically following **(MacFaddin , 2000)** tests . **2.2.2. Serotyping of E. coli isolates**

The Serotyping of E. coli isolates was applied in the Reference Laboratory for Veterinary Quality Control of Poultry Production, according to **(Ewing, 1986),** by using slide agglutination technique with commercially available polyvalent and monovalent anti *E.coli* O and K sera (Test Sera Enteroclon, Anti –Coli, SIFIN Berlin, Germany).

2.3. Antimicrobial susceptibility testing.

Phenotypic antibiotic susceptibility pattern was studied using six antibiotic groups, using most frequent ten chemotherapeutic agents representing in the field which performed by antibiotic disk diffusion method and the results were interpreted according to the criteria recommended **(CLSI,2017)** for antimicrobial susceptibility testing.

2.4. Conventional PCR technique.

DNA extraction and PCR amplification:

DNA was extracted according to QIAamp DNA mini kit instructions from isolates, details oligonucleotide primer which supplied from Metabion (Germany) and PCR conditions are listed in table 1. PCR reaction (25 µl) contained 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara, Japan), 1 µl of 20 pmol concentration of each primer, 4.5 µl of water, and 6 µl of the DNA template. PCR reactions were performed in Applied Biosystems 2720 Thermal Cycler. Each PCR product was loaded in a separate well in 1.5% agarose gel, then photographed and analyzed using a gel documentation system (Alpha Innotech, Biometra, Germany) through its computer software.

Table 1: Primers sequences and amplicon sizes of target genes.

Target gene	Function of target gene	Primers sequences	Amplified segment (bp)	Reference
***pho*A**	**Conserved virulence gene**	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	**(Hu *et al.*, 2011)**
***iro*N**	**Iron acquisition virulence gene**	TCGCTAACTGATCCTATG CTGCACTGGAAGAACTGTTCT	847	**(Ewers *et al.*, 2007)**
***omp*T**	**Outer membrane protein virulence gene**	AGCTATCGCGATTGCAGTG GGTGTTGCCAGTAACCGG	919	**(Ewers *et al.*,2007)**
iss	**Increased serum survival virulence gene**	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266	**(Yaguchi *et al.*, 2007)**
***aad*A1**	**Aminoglycosides resistant gene**	TGATTTGCTGGTTACGGTGAC CGCTATGTTCTCTTGCTTTTG	284	**(Sabarinath *et al.*, 2011)**
***tet*A**	**Tetracycline resistant gene**	CCTTATCATGCCAGTCTTGC ACTGCCGTTTTTTCGCC	576	**(Sabarinath *et al.*,2011)**
***sul*I**	**Trimethoprime sulfamethoxazole resistant gene**	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	**(Ibekwe *et al.*, 2011)**
***bla*CTX-M**	**β-Lactams resistant gene**	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	**(Archambault *et al.*, 2006)**
***qnr*S**	**Quinolones resistant gene**	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417	**(Robicsek *et al.*,2006)**
***erm*B**	**Macrolides resistant gene**	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	425	**(Schlegelova *et al.*,2008)**

2.4. Experimental design.

A strategy adopted by experimental application of *Neem* leaves extracts (NLE) for prevention and treatment of colibacillosis on one day old SPF chicks and evaluation of morbidity and mortality rates ,pathological changes on infected of chicks in addition to measuring and evaluation of different haematological parameters, clinic pathological and immune response on infected chicks. Eighty-one day-old SPF chicks reared in animal house under strict hygienic conditions, feed and water, these chicks were divided into five groups (A, B, C, D and E) containing 16 birds each group, more bacteriological, histopathological and hematological studies were done after induction of the infection **(Table 2).**

Ethical approval

The experimental study was done according to an approved protocol by the Ethical Committee of

the Animal Health Research Institute(AHRI),ARC, Ministry of Agriculture, Giza, Egypt.

Infected bacterial strain: selected multidrug resistant *E.coli* O_{119} with concentration 1.0×10^8 CFU/ 0.5ml orally at 5th day **(Ibrahim, 2012).**

Table 2: Experimental design of different groups

Group	No. of experimental chicks	Treatment
Group (A)	16	Control negative
Group (B)	16	Control positive Infected bacterial strain (pervious dose)
Group (C)	16	10% *Neem* leaves extract in water at (1st till 21th day) + Infected bacterial strain (pervious dose)
Group (D)	16	Infected bacterial strain (pervious dose) + After 8 day post infection (Amoxicillin antibiotic) 10 mg/kg.B.Wt for 8day
Groups (E)	16	10% *Neem* leaves extract in water at (1st till 21th day) + Infected bacterial strain (pervious dose) + After 8 day post infection (Amoxicillin antibiotic) 10 mg/kg.B.Wt for 8day

Preparation of *Neem* leaves extracts (NLE)

Neem leaves were shade dried and powdered, the aqueous extract was prepared from powdered *Neem* leaves. One hundred gram *Neem* leaves powder was boiled in 1 L water / 15 min, then after straining the extract added to drinking water to make the volume of 1L **(Raheja,2004).**

2.6. Histopathological examination.

Tissues specimens from liver, heart, air sac, lung, kidney, duodenum, cecum, bursa of fabricius, spleen and pancreas which were fixed with 10% buffered formalin with dehydrated in alcohols, embedded in paraffin, cleared in xylene, and sectioned at 5 µm, the sections were stained with H&E which examined for any pathological changes according to **(Suvarna *et al.*,2018)**,the histopathological lesion grading was calculated by description of histomorphological changes in 5 fields per section for each examined organ according to **(Katherinen *et al.*, 2013).**

2.7. Hematological investigation.

At the end of treatment, two blood samples were taken from wing vein of 5 birds of each group:

First blood samples was collected in EDTA tubes for estimation of total erythrocyte count ,hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), MCH ,MCHC and total differential leukocyte count (TLC), according to **(Schalm *et al.*, 1975).**

Second blood samples was collected without anticoagulant in clean dry centrifuge tube to obtain clear serum for estimation of alanine amino transferase (ALT) and aspartate amino transferase (AST) **(Reitman and Frankel, 1975),** creatinine **(Slot, 1965)** uric acid **(Sanders and Pasman, 1980),** catalase activity **(Sinha, 1972)** , IL6 **(Unvera and Mcallister, 2018)** and IgA and IgM **(Giambrone and Ronald, 1986).**

2.8. Statistical analysis.

The pervious data were analyzed statistically using SPSS program version (23), the standard deviation and averages of different groups were calculated, using ANOVA test for comparison between the different groups and followed by post hoc test using Duncan multiple range (DMR) test for comparisons between means of groups. The means with same letter in each column are not significant, while different letter from each other significant at the 5-percent probability level (p value at 0.05), as described by **(Tamhan and Dunlop,2000).**

3. Results

3.1. Bacteriological and serotyping finding.

In the present study, out of 200 samples, one hundred four isolates were positive for *E.coli* (52%). Serotyping of E.*coli* isolates revealed their distribution in eight serotypes, high incidence ratio was O_6 (23%), followed by O_{119}, O_2, O_{149} (15.4%) for each, whereas O_{124}, O_{91}, O_{128}, O_{55} had the lowest incidence in *E.coli* strains (7.7%), **(Table 3).**

Table 3: Serotypes distribution of *Escherichia coli* isolates between broiler chickens:

Serotype	No. of isolates(%)
O6	24/104(23%)
O119	16 /104(15.4%)
O2	16 /104(15.4%)
O146	16 /104(15.4%)
O124	16 /104(7.7%)
O91	8 /104(7.7%)
O128	8 /104(7.7%)
O55	8 /104(7.7%)

3.2. Studying the phenotypic antimicrobial resistance profiles of isolates.

The highest antimicrobial resistance levels were found against amoxicillin (100%), followed by apramycin, ampicillin and tetracycline (84.6%) for each, then streptomycin and erythromycin in percentage of (76.9%), while the lowest resistance was against doxycycline (53.8%), **(Table 4).**

3.3. Confirmation of Isolates by PCR, figure (1).

3.3.1. Investigation of genotypic virulence attributes of isolates by PCR.

Ten E.*coli* isolates with multidrug resistance phenotypic attributes were randomly selected and tested by PCR, **(Table 5),** the results revealed that all 10/10(100%) of the tested E.*coli* isolates were positive for each (*pho*A and *iss*) genes as demonstrated in **(Figs.1A and1C)**, while the acquisition for (*iro*N and *omp*T) virulence genes were detected with percentage (80% for each) **(Figs.1B and 1D)**.

Table 4: Phenotypic resistance profiles:

Chemotherapeutic Group	Chemotherapeutic Agents / Disc Potency Mg/disc	No. of resistant Strains (%)
Aminoglycosi-des	Streptomycin (10µg)	80(76.9%)
	Apramycin (15 µg)	88(84.6%)
Tetracyclines	Tetracycline (30µg)	88(84.6%)
	Doxycycline (30µg)	56 (53.8%)
β-Lactams	Ampicillin (10ug)	88(84.6%)
	Amoxicillin (25 µg)	104(100%)
Diaminopyri-midine	Trimethoprim (10 µg)	72 (69.2%)
Quinolones	Norfloxacin (10ug)	48 (46.2%)
	Nalidexic acid (30 µg)	64 (61.5%)
Macrolides	Erythromycin (50 µg)	80(76.9%)

3.3.2. Investigation of genotypic resistant attributes of isolates by PCR.

β- lactamases resistant genotypic attributes was tested by PCR for the presence of (*bla*CTX-M) gene 10/10 (100%) as shown in (**Fig.1F)**, aminoglycosides and quinolones resistant were tested for (*aad*AI and *qnr*S) genes, which were confirmed in 9/10 (90% for each) of the tested E.*coli* isolates as shown in **(Figs.1E and 1I)**, followed by macrolides and tetracycline resistance (*erm*B and *tet*A) genes which were detected in 8/10 (80% for each) of isolates as shown in **(Figs.1H and 1J),** while resistance genes for trimethoprime sulfamethoxazole (*sul*I) genes was revealed 7/10 (70%) as shown in **(Fig 1G).**

Table 5: PCR amplifications results of different virulence and resistant genes of isolates:

Isolates no.	Serotype	Virulence genes				Resistant genes					
		***pho*A**	***iro*N**	***iss***	***omp*T**	***aad*AI**	***bla*CTX-M**	***erm*B**	***sul*I**	***tet*A**	***qnrS***
1	O6	+	+	+	+	+	+	+	+	+	+
2	O119	+	+	+	-	+	+	+	+	-	+
3	O6	+	+	+	+	+	+	+	+	+	+
4	O119	+	+	+	+	+	+	+	-	+	+
5	O2	+	+	+	-	-	+	+	-	+	+
6	O146	+	+	+	+	+	+	+	+	+	+
7	O124	+	+	+	+	+	+	+	+	+	+
8	O91	+	-	+	+	+	+	-	+	+	+
9	O128	+	+	+	+	+	+	+	+	+	+
10	O55	+	-	+	+	+	+	-	-	-	-
Total (%)		10/10 (100%)	8/10 (80%)	10/10 (100%)	8/10 (80%)	9/10 (90%	10/10 (100%)	8/10 (80%)	7/10 (70%)	8/10 (80%)	9/10 (90%)

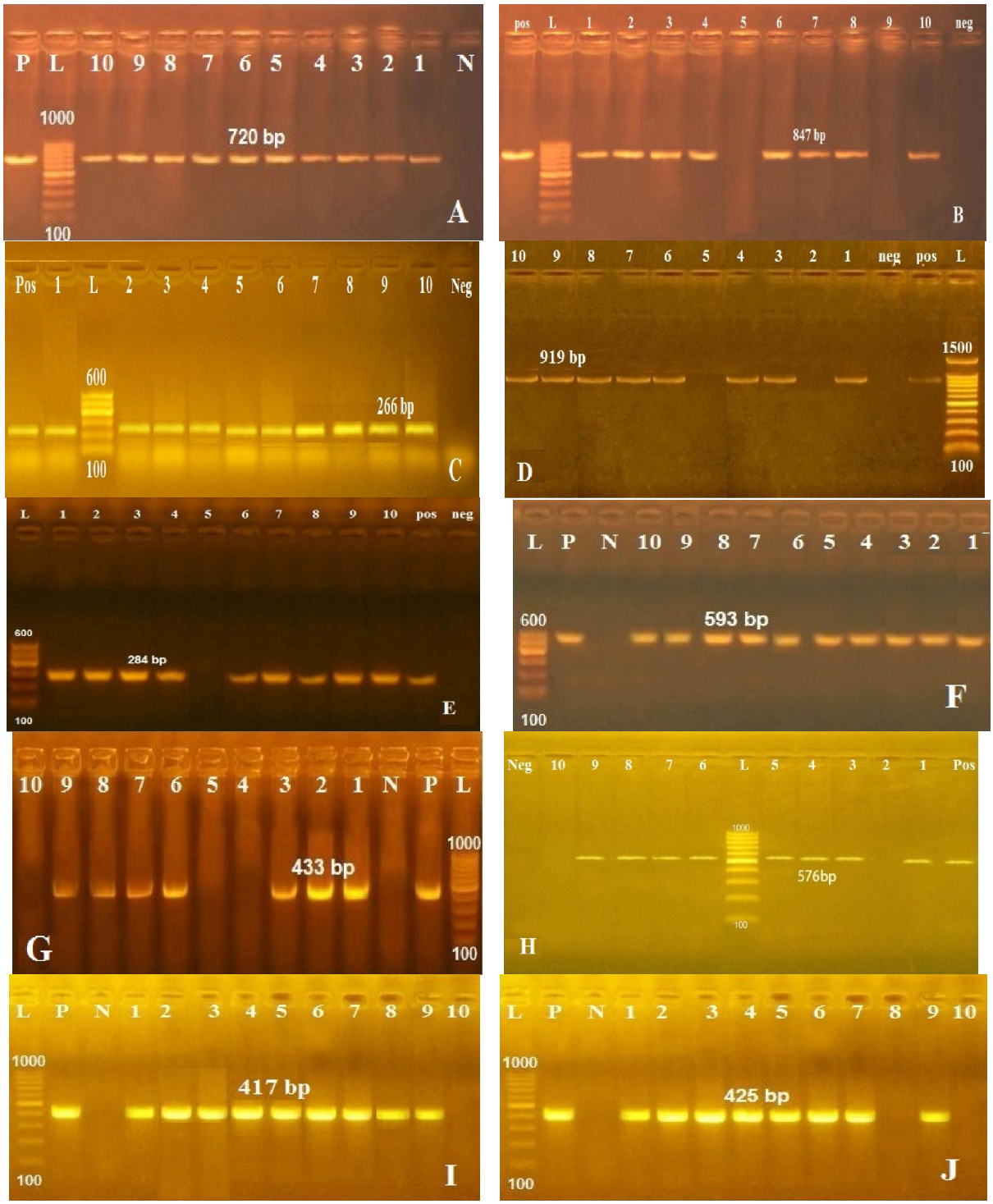

Fig. (1): Agarose gel electrophoresis of PCR amplified products, Lane (L): DNA molecular size marker, lane (P): positive control , lane (N): negative control, **(A)**: lane 1-10 positive for *pho*A at 720bp , **(B)**: lane 5,9 negative for *iroN* at 847bp, **(C)**: lane 1-10 positive for *iss* at 266bp, **(D):** lane 2,5 negative for *ompT* at 919bp, **(E)**: lane 5 negative for *aadAI* at 284bp, **(F):** lane 1-10 positive for *blaCTX*-M at 593bp, **(G):** lane 4,5,10 negative for *sul*I at 433bp, **(H):** lane 2,10 negative for *tetA* at 576bp, **(I):** lane 1-9 positive for *qnrS* at 417bp, **(J):** lane 8,10 negative for *ermB* at 425bp.

3.4. Histopathological finding of experimental chicks.

Clinical signs and gross lesions:

Clinically.

The birds infected with *E. coli* isolate O_{119} 0.5 ml/10^8 CFU after 8 days of infection showed weakness, dropped wings, inability to move, greenish to brownish diarrhea with or without respiratory signs.

Grossly,**(Fig.2)**:

In infected chickens (GB), the most relevant lesions of colibacillosis were fibrinous pericarditis, fibrinous perihepatitis **(Fig.2a),** and airsacculitis **(Fig.2b)** which mean covering the heart, liver, and air sacs with thick fibrinous membrane accompanied with caseated materials. Some of these fibrinous membranes were easily separated and others adhered with the serosal surface. The air sacs were thicken, opaque, and yellowish. The internal organs as liver, heart, lung, spleen, kidney, pancreas appeared congested. Bursa appeared atrophy in most necropsied cases. The two ceci of most birds filled with greenish or brownish content with gas **(Fig.2c)**, the mortality rate was (75%).

The *Neem* treated chicks before and after infection (GC) showed congested hepatic parenchyma and internal organs (**Fig.2d).** However, the neem-treated group before infection and antibiotic-treated after infection (GE) showed the best results of improving healthy state of chicks followed by antibiotic-treated group after infection (GD)which showed normal health condition of affected birds **(Fig.2e).**

Liver, (Fig.3):

Control positive group (GB) showed fibrinous membrane in the hepatic peritoneal cavity which composed from dilated capillaries, fibrin exudate, inflammatory cells and caseated materials. Moreover, congested hepatic vasculatures with presence of fibrinous thrombi within some vessels were also detected .Degenerative and necrotic changes were seen in large number of hepatocytes. The most frequently inflammatory cells infiltrations as heterophils, lymphocytes, macrophages, and plasma cells were seen in the hepatic peritoneal sac, **(Fig.3a)**.While, group (C) showed mild degenerative changes within certain hepatocytes **(Fig.3b)**. Group (D) showed dilated some blood vessels and sinusoids **(Fig.3c).**While group (E) showed apparently normal hepatic acini and hepatic vasculatures**.** However, dilated some hepatic sinusoids were also detected, **(Fig.3d)**.

Heart, (Fig.4):

Control positive group (GB) showed fibrinous pericarditis which represented by adhesions between the pericardium and epicardium by fibrin threads, inflammatory cells infiltration and caseated debris. Interstitial myocarditis were also detected which consisted of inflammatory cells infiltration between degenerated myocardial fibers **(Fig.4a).** While group (C) showed mild thickening of the pericardium by few amount of fibrin and caseated debris beside presence of interstitially inflammatory cells infiltrtions **(Fig.4b).** Group (D) showed mild interstitial myocarditis and degenerative changes of some cardiac muscle fibers **(Fig.4c).**While group (E) showed apparently normal cardiomyocytes and pericardium. But some myofibers appeared degenerated. **(Fig.4d).**

Air sacs, (Fig.5) :

Control positive group (B**)** showed thickened membrane which represented by caseated debris intermixed with heterophils, macrophages, plasma cells and lymphocytes in addition to dilated capillaries and fibrin exudates **(Fig.5a).** While group (C)showed mild thickening of the membranes by dilated capillaries and serofibrinous exudate **(Fig.5b).**Group (D) showed reduced thickness of the inflamed membrane **(Fig.5c).**While group (E) showed apparently normal air sacs with mildly dilated capillaries **(Fig.5d).**

Lung, (Fig.6):

Control positive group (B) showed congestion in pulmonary blood vessels, infiltrations of heterophils, macrophages and lymphocytes in peribronchial alveoli and wall of bronchus. Fibrinous and caseated exudate was seen within some bronchi and parabronchial lumina **(6.a).** While group (C) showed serofibrinous exudate within parabronchial lumina as well as mild congested pulmonary blood vessels **(Fig.6b).** Group (D) showed mild dilated blood vessels beside inflammatory cells infiltration within some air vesicles **(Fig.6c).**While group (E) showed apparently normal cytoarchitectures of pulmonary tissue with presence aggregations of inflammatory cells in some areas, **(Fig.6d).**

Kidney, (Fig.7) :

Control positive group (B**)** showed congestion of renal blood vessels and necrosis of some renal tubules**. (Fig7a)**.While group (C) showed dilated some renal blood vessels with degenerative changes of some renal tubular epithelium **(Fig7b),** also Group

(D) have degenerative changes in some renal tubular epithelium. **(Fig7c)**.While group (E) showed apparently normal glomerular and tubular structures**, (Fig.7d)**.

Duodenum , (Fig.8) :

Control positive group (B) showed duodenitis which represented by numerous leukocytic infiltrations within submucosa and desquamated epithelium within intestinal lumen admixed with necrotic debris **(Fig.8a).**While group (C) showed desquamated epithelial lining villi in addition to submucosal inflammatory cells infiltrations **(Fig.8b).** Group (D) showed numerous inflammatory cells infiltrtions within submucosa **(Fig.8c).**While group (E) showed apparently normal intestinal layers with numerous goblet cells on villi. **(Fig.8d).**

Cecum , (Fig.9) :

Cecitis were demonstrated as dilated submucosal blood vessels, desquamated epithelium lining mucosa and leukocytic infiltrations within cecal wall in group (B), **(Fig.9a).** While group (C) showed heavy submucosal inflammatory cells infiltrations **(Fig.9b).**Group (D) showed moderately inflammatory cells infiltrations within submucosa **(Fig.9c).** While normal intestinal layers with few number of leukocytic infiltration in submucosa in group (E), **(Fig.9d).**

Bursa of Fabricius, (Fig.10):

Control positive group (GB) showed depleted some lymphoid follicles and caseated material with leukocytic infiltrations within other follicles**. (Fig.10a)**. While group (C) showed mild interstitial edema between follicles and caseated material in some follicles **(Fig.10b).** Group (D) showed apparently normal lymphoid follicles with mildly depleted some follicles **(Fig.10c)**. While normal lymphoid populations were showed in group (E), **(Fig.10d).**

Spleen, (Fig.11) :

Control positive group (B**)** showed lymphoid depletions with fibrinous and caseated exudate in some follicles. Moreover, thickening of covering serosal membrane by fibrin exudate, inflammatory cells infiltrations was seen **(Fig.11a).** While group (C) showed depletion of some white pulp lymphoid aggregations **(Fig.11b).**Group (D&E) showed proliferation of reticuloendothelial cells of the spleen which indicating ameliorative effect, **(Fig.11c and11d).**

Pancreas , (Fig.12):

Control positive group (B) showed hyperplastic mesothelium lining serosal membrane with thickening of the membrane by fibrin threads and leukocytic infiltrations, degenerative and necrotic changes within pancreatic acini were also detected. **(Fig.12a).**While group (C) showed degenerative changes of some pancreatic acini beside multiple foci of inflammatory cells infiltration, **(Fig.12b).** Group (D) showed apoptotic some epithelial lining pancreatic acini, **(Fig.12c).** While group (E) showed apparently normal pancreatic tissue with presence degenerative changes of epithelial lining some acini**, (Fig.12d).**

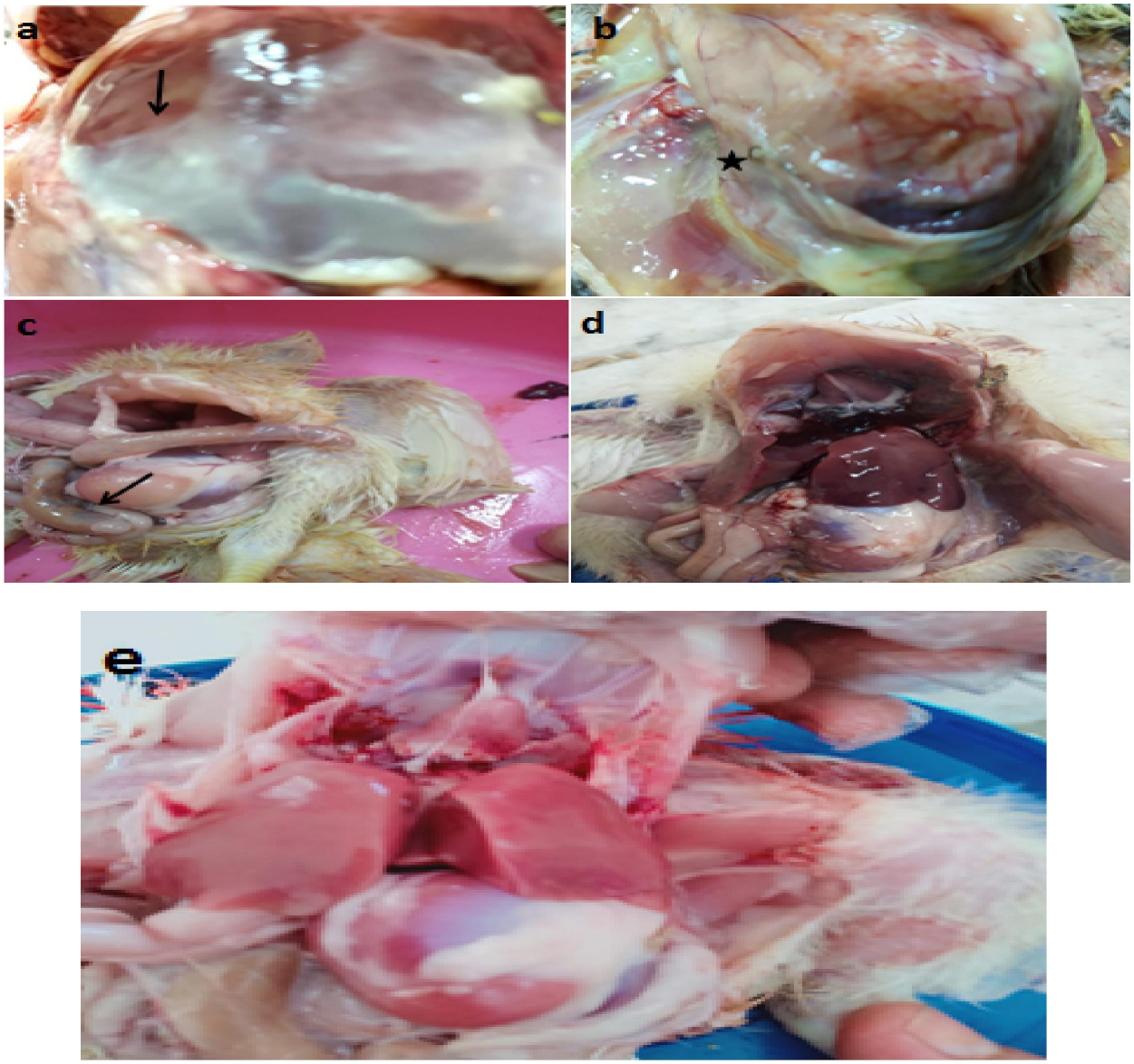

Fig.2: Gross picture of group (B) showed **(a)** Fibrinous perihepatitis **(arrow)**, **(b)** Airsaculitis **(star)**, **(c)** Gases filled cecum **(arrow), (d)** Group (C) showed congested hepatic parenchyma and internal organs, **(e)** Group (D,E) showed apparently normal internal organs.

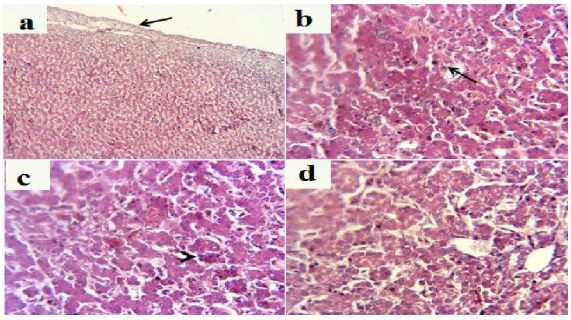

Fig. 3: photomicrograph of liver **H&E X 400** showing:
(a): Group **(B)** showed fibrinous membrane in the hepatic peritoneal cavity **(arrow)** and degenerative and necrotic changes in large number of hepatocytes , **(b):** Group (C) showed mild degenerative changes within certain hepatocytes **(arrow), (c):** Group (D) showed dilated some sinusoids **(arrowhead)**, **(d):** Group (E) showed apparently normal hepatic acini and dilated some hepatic sinusoids.

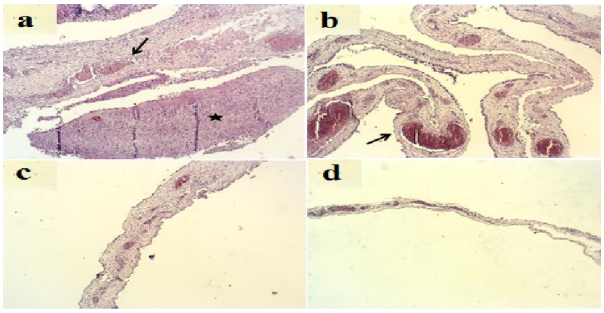

Fig .5: photomicrograph of air sac **H&E X 100**showing:
(a): Group **(B)** showed thickened membrane by caseated debris intermixed with inflammatory cells **(star)** in addition to dilated capillaries **(arrow)** and fibrin exudates,**(b):** Group (C) showed mild thickening of the membranes by dilated capillaries **(arrow)** and serofibrinous exudate**, (c):** Group (D) showed reduced thickness of the inflamed membrane.**, (d):** Group (E) 16 days PI showing apparently normal air sacs with mildly dilated capillaries**.**

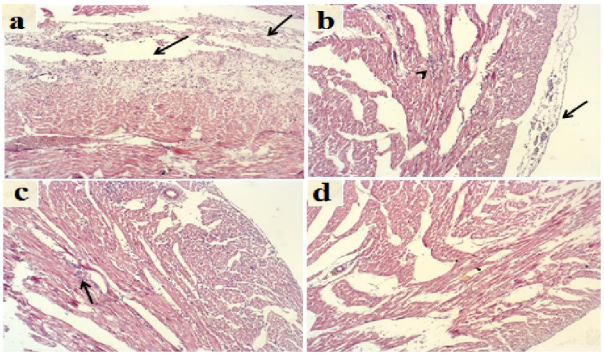

Fig.4: photomicrograph of heart **H&E X 100** showing:
(a): Group **(B)** showed fibrinous pericarditis **(arrows)** and interstitial myocarditis**,(b):** Group (C) showed mild thickening of the pericardium by few amount of fibrin and caseated debris **(arrow)** beside presence of interstitially inflammatory cells infiltrations **(arrowhead), (c):** Group (D) showed mild interstitial myocarditis **(arrow), (d):** Group (E) showed apparently normal cardiomyocytes and pericardium with presence some degenerated myofibers.

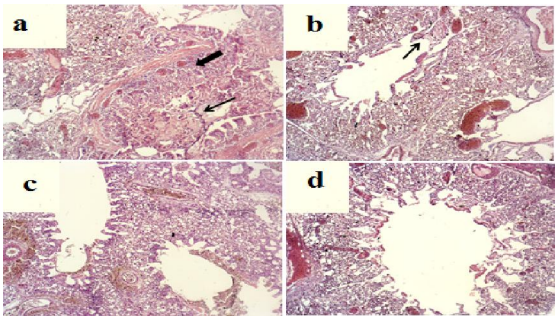

Fig .6: photomicrograph of lung **H&E X 100**showing:
(a): Group (B) showed inflammatory cells infiltrtaions in the wall of bronchus **(thick arrow)** beside fibrinous and caseated exudate within some bronchi and parabronchial lumina **(arrow) , (b)**: Group (C) showed serofibrinous exudate within parabronchial lumina **(arrow)** as well as mild congested pulmonary blood vessels, **(c)**: Group (D) showed mild dilated blood vessels beside inflammatory cells infiltration within some air vesicles, **(d)**: Group (E) showed apparently normal cytoarchitectures of pulmonary tissue with presence aggregations of inflammatory cells in different areas**.**

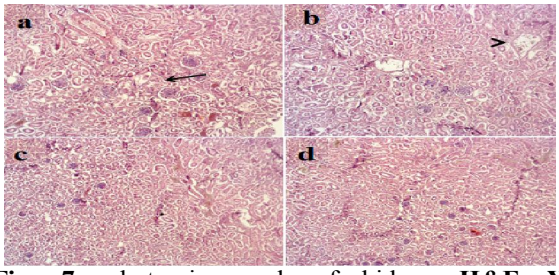

Fig .7: photomicrograph of kidney **H&E X 100**showing:
(a): Group (B) showed necrosis of some renal tubules **(arrow)**, **(b):** Group (C) showed dilated some renal blood vessels **(arrowhead)** beside degenerative changes of some renal tubular epithelium, **(c):** Group (D) showed degenerative changes in some renal tubular epithelium, **(d):** Group (E) showed apparently normal glomerular and tubular structures**.**

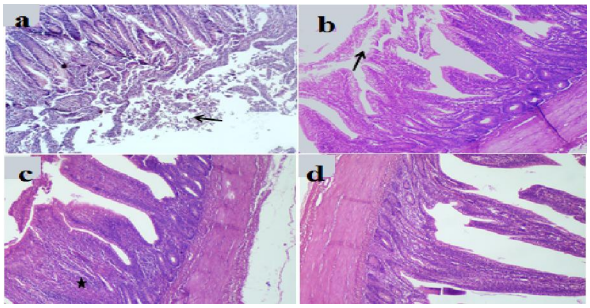

Fig .8: photomicrograph of duodenum **H&E X 100**showing:
(a): Group (B) showed desquamated epithelium within duodenal lumen admixed with necrotic debris **(arrow)** and numerous leukocytic infiltrations within submucosa, **(b):** Group (C) showed desquamated epithelial lining villi **(arrow)** in addition to submucosal inflammatory cells infiltrations, **(c):** Group (D) showed numerous inflammatory cells infiltrations within duodenal submucosa **(star)**,**(d):** Group (E) showed apparently normal intestinal layers with numerous goblet cells on villi**.**

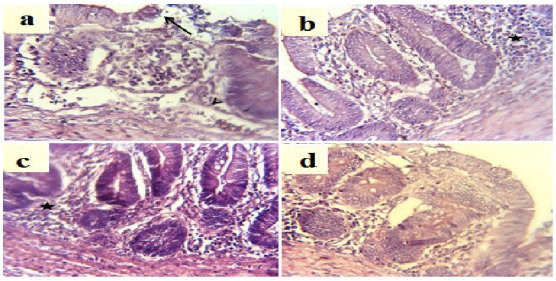

Fig.9: photomicrograph of cecum **H&E X 400** showing:
(a): Group (B) showed dilated submucosal blood vessels **(arrowhead),** desquamated epithelium lining mucosa **(arrow)** and leukocytic infiltrations within cecal wall., **(b):** Group (C) showed heavy submucosal inflammatory cells infiltrations **(star), (c):** Group (D) showed mild inflammatory cells infiltrations within cecal submucosa **(star),(d):** Group (E) showed apparently normal intestinal layers with few number of leukocytic infiltration in submucosa**.**

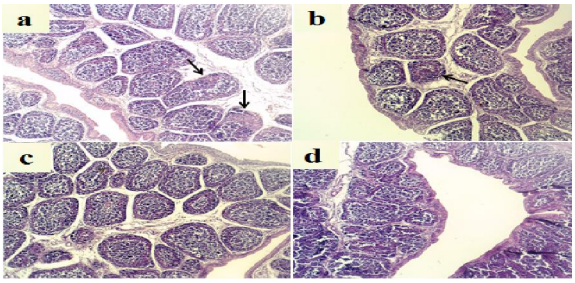

Fig .10: photomicrograph of Bursa of Fabricius **H&E X 100**showing:
(a): Group (B) showed depleted some lymphoid follicles with caseated material **(arrows), (b):** Group (C) showed mild interstitial edema between follicles and caseated material in some follicles **(arrow)**., **(c):** Group (D) showed apparently normal lymphoid follicles with mildly depleted some follicles, **(d):** Group (E) 16 days PI showing apparently normal lymphoid populations**.**

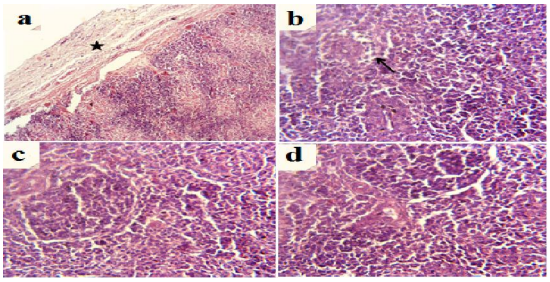

Fig. 11: photomicrograph of spleen **H&E X 400** showing:
(a): Group (B) showed lymphoid depletions in some follicles and thickening of covering serosal membrane **(star)** , **(b):** Group (C) showed mild interstitial edema between follicles and caseated material in some follicles **(arrow)**,**(c):** Group (D) showed apparently normal white pulp lymphoid populations., **(d):** Group(E) showed apparently normal white pulp and proliferation of reticuloendothelial cells**.**

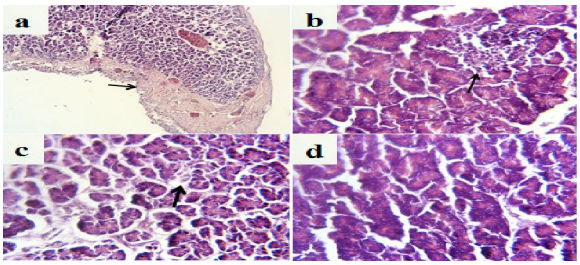

Fig.12: photomicrograph of pancreas **H&E X 400** showing:
(a): Group (B) showed thickening of the pancreatic serosal membrane by fibrin threads and leukocytic infiltrations **(arrow).**,**(b):** Group (C) showed degenerative changes of pancreatic acini beside foci of inflammatory cells infiltration **(arrow)**., **(c):** Group (D) showed apoptotic some epithelial lining pancreatic acini **(arrow)**,**(d):** group (E) showed apparently normal pancreatic tissue with degenerative changes of some epithelial lining acini**.**

3.5. Hematological results.

There was a significant decrease in RBCs count, Hb concentration and PCV% in GB,GC and GD, as compared with control group GA the most significant decrease in infected group GB. MCV was increased in group B, C and D compared with control group. MCH and MCHC were not significantly changed in all groups compared with control group, all examined parameters in group E not significantly changed compared with the control group, **Table (7).**

LeuKogram **Table (8),** there was a significant increase in TLC ,heterophils and monocytes in groups B, C and D as compared with control group A, whereas lymphocytes was decreased significantly in groups B,C and D. Eosinophil and basophils were not significantly changed compared with control group.

Total and differential leukocytic values were not significantly changed in group E compared with control group.

3.6. Biochemical Results.

A significant increase in ALT ,AST enzymes ,uric acid ,creatinine , IgA , IgM and IL6 in groups B ,C and D compared with the control group A, the highest increase was in group B, while a significant decrease in catalase enzyme in groups B, C and D compared with control group A.

All examined biochemical parameters in group E were not significantly changed compared with control group, **Table (9).**

Table 6: Summarized the lesion scores of many histopathological lesions within different organs among different groups:

Organ	Lesions	(GA)	(GB)	(GC)	(GD)	(GE)
Liver	Fibrinous perihepatitis	-	+++	-	-	-
	Degenerative and necrotic changes	-	++	++	+	-
	Inflammatory cell infiltrations	-	++	+	+	+
	Congested hepatic blood vessels	-	++	+	+	-
	Thrombi within hepatic vasculatures	-	+++	+	+	-
Heart	Fibrinous pericarditis	-	+++	++	+	-
	Interstitial myocarditis	-	++	+	+	-
	Degenerated cardiomyocytes	-	++	+	+	+
	Congested blood vessels	-	+++	-	-	-
Air sac	Caseated materials	-	+++	-	-	-
	Fibrinous exudate	-	+++	+	+	-
	Dilated capillaries	-	++	++	+	-
	Inflammatory cells	-	+++	+	+	+
Lung	Bronchial exudate	-	++	+	-	-
	Inflammatory cells infiltrations	-	+++	+	+	-
	Congested pulmonary blood vessels	-	+++	++	+	+
Kidney	Degenerated and necrotic changes	-	++	+	+	-
	Congested renal blood vessels	-	++	++	+	-
Duodenum	Desquamated villus epithelium	-	+++	++	+	-
	Leukocytic infiltrations	-	+++	+	+	-
Cecum	Desquamated mucosal epithelium	-	+++	+	+	-
	Leukocytic infiltrations	-	++	++	+	+
	Dilated submucosal blood vessels	-	+++	+	+	-
Bursa	Lymphoid depletions	-	++	-	-	-
	Caseated materials within follicles	-	++	-	+	-
Spleen	Thickening seosal membrane	-	++	+	+	-
	Lymphoid depletion	-	++	+	-	-
	Fibrinous thrombi within vasculatures	-	+++	++	+	-
Pancreas	Fibrinous exudate on capsule	-	+++	+	+	-
	Foci of inflammatory cells infiltrtion	-	++	++	+	-
	Degenertive changes of acini	-	++	+	+	-

(GA): Control –ve, **(GB)**: Control +ve**, (GC):** neem–treated group, **(GD):** antibiotic-treated group after infection, **(GE)**: neem-treated group, **(-)** No altrerations**, (+)** Mild , **(++)** Moderate, **(+++)** Severe alterations.

Table 7: Hematological results between different experimental groups. (Mean±SD) N=5

Groups	RBCs 10^6/cm	Hb gm/dl	PCV %	MCV fl	MCH pg	MCHC gm/dl
G A	3.44 a ± 0.67	10.42 a ± 0.07	36.38 a ± 0.40	105.67 c ± 8.72	30.29 ± 4.78	28.64 ± 3.70
G B	2.32 d ± 0.58	8.10 d ± 0.05	28.66 d ± 0.39	123.53 a ±11.81	34.91 ± 5.09	28.26 ± 5.11
G C	2.75 c ± 0.55	8.74c ± 0.09	30.94 c ± 1.09	112.51 b ± 10.56	31.78 ± 5.12	28.25 ± 2.78
G D	3.12 b ± 0.51	9.48 b ± 0.11	33.60 b ± 0.37	107.69 c ± 9.87	30.38 ± 6.15	28.21 ± 4.06
G E	3.48 a ± 0.77	10.46 a ± 0.12	35.67 a ± 0.55	102.76 c ± 10.00	30.06 ± 5.90	29.25 ± 6.11
Pr>f	0.012	0.025	0.042	0.247	0.218	0.180
Significance	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**

Table 8: Lukogram of different experimental groups. (Mean ± SD) N=5

Groups	TLC 10^3/cm	Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
G A	14.52 d ± 0.24	7.36 a ± 0.11	4.54 d ± 0.25	1.84 d ± 0.25	0.43 ± 0.01	0.35 ± 0.02
G B	28.92 a ± 0.35	4.64 d ± 0.12	16.47 a ± 0.16	7.04 a ± 0.16	0.43 ± 0.01	0.34 ± 0.02
G C	24.14 b ± 0.49	5.32 c ± 0.07	12.13 b ± 0.29	5.96 b ± 0.12	0.45 ± 0.01	0.31 ± 0.02
G D	19.28 c ± 0.46	6.78 b ± 0.04	8.04 c ± 0.34	3.74 c ± 0.10	0.41 ± 0.02	0.31 ±0.03
D E	14.62 d ± 0.23	7.76 a ± 0.25	4.30 d ± 0.10	1.82 d ± 0.23	0.41 b ± 0.02	0.32 ± 0.01
Pr>f	0.760	0.383	0.740	0.072	0.002	0.011
Significance	**No**	**No**	**No**	**No**	**Yes**	**Yes**

Table 9: Some biochemical parameters of different experimental groups. (Mean ± SD) N=5

Groups	ALT U/L	AST U/L	U.acid mg/dl	Creat mg/dl	Catalase ng/ml	IgA ng/ml	IgM ng/ml	IL6 Pg/ml
G A	10.64 b ± 0.48	165.96 c ± 1.87	2.33 c ± 0.03	0.43d ± 0.07	0.62 a ± 0.03	0.44 a ± 0.53	2.32 c ± 0.03	1.42d±0.05
G B	31.21 a ± 1.77	297.74 a ± 14.26	4.43 a ± 0.15	0.87a ±0.01	0.24 d ± 0.07	0.77 c ± 0.01	4.43 a ± 0.15	5.14 a ±0.12
G C	18.89 c± 1.42	216.51 b ± 10.74	3.15 b ±0.29	0.48c ± 0.08	0.36c ± 0.09	0.58 b ± 0.01	3.16 b ± 0.29	1.97c ±0.10
G D	14.68 b ± 0.94	189.33 c ± 3.38	3.20 b ± 0.17	0.59 b ± .o1	0.44 b ± 0.08	0.56 b ± 0.01	3.20 b ± 0.17	3.90 b ±0.11
G E	12.54 b ± 0.54	170.68 c ± 0.92	2.7b c ± 0.07	0.46 d ± 0.05	0.60a ± 0.03	0.46 a ± 0.01	2.77 bc ± 0.07	1.51d ±0.02
Pr > f	0.006	0.000	0.000	0.001	0.000	0.461	0.000	0.000
Significance	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**yes**

4. Discussion

The emergence of multidrug antibiotic-resistant bacteria (MDR) has been as a result of common and frequent use of antibiotics in veterinary medicine as well as in industry and agriculture. In this investigation, high recovered rate of *E.coli* isolates (52%) was recorded, similar results were reported by **(Soad *et al.*,2017)** who isolated in percentage of 60%. Serotyping of isolates confirmed the identification of O_6 (23%) and O_2, O_{119}, O_{149} (15.4%) which were the most common serotypes, these result were agreement with that recorded by **(Hedican *et al.*, 2009).**

One health approach unanimously confirm the importance relation between humans , animals and the environment which following WHO guidelines to use medically important antimicrobials in food producing animals was help on limiting the problem of antimicrobial-resistant and food safety concerns and how to manage **(WHO,2017).** Several recent studies have documented antibiotic resistance especially among APEC strains **(Khan *et al.*,2002).** These studies were in matching with our study which recorded high levels of resistance against some antimicrobial agents including: Amoxicillin (100%), followed by apramycin, ampicillin and tetracycline (84.6%) for each, then streptomycin and erythromycin (76.9%), these results were agreement with **(Abd El Tawab *et al.*,2015).**

PCR depend on genetic determinants rather than the phenotypic traits as the culture does, which allowing the identification of different species instead if gone not detected by using only the classical phenotypic dependent culture assay **(Alnahass *et al.*,2016)**. Furthermore, highly specific, it is simple, can provide results on the same day of sample for evaluation, which is a potential advantage in epidemiological studies and outbreaks **(Shanta *et***

***al*.,2001).** Consequently, studying the distribution of virulence genes could contribute in clarifying the pathogenesis of diseases in broiler chickens.

In our study, a general *E.coli (pho*A) gene was detected in all isolates (100%) which confirmed presence of *E. coli* species, the results agree with **(Hu *et al*.,2011)** who detected (95%) of *E. coli* isolates had *(pho*A) gene.

With regard to the prevalence of virulence genes among *E. coli* isolates, it was recorded that (100%) of *E. coli* isolates have (*iss* gene), this result is consistent with a previous report by **(Ewers *et al*., 2007)** who detected (*iss* gene) in APEC with higher percentages (95.5%) which have important role in *E. coli* pathogenicity with a potential target for developing novel therapeutics and prevention strategies.

Studying the genotypic virulence attributes of *(iro*N gene) which associated with *E. coli* virulence especially in bacteria causing septicemia which use these system to get the heme molecule to survive in the host and aquatic habitat, the results revealed the detection of *(iro*N gene) in 8/10 (80%) of the tested multidrug resistance *E.coli* isolates, similar result was recorded by **(Master *et al*.,2011)** who detected in (90%) of tested isolates.

The recorded results revealed also that 8/10(80%) of *E. coli* isolates were positive for *(omp*T gene) that is responsible for the production of outer membrane protease of bacteria and attachment to the host, this result is consistent with **(Jeonq *et al*.,2012)** who documented the prevalent carriage of *(omp*T gene) in APEC strain with percentages (94.1%).

According to phenotypic attributes of isolates, PCR was applied for studying the antimicrobial genotypic characters through the detection of β-Lactams resistance gene (*bla*CTX-M), all ten MDR *E.coli* isolates were positive 10/10 (100%), the result agree with **(Bass *et al*.,1999)** who detected (100%) of isolates carried this gene.

Moreover, aminoglycosides and quinolones resistance genes among ten MDR *E. coli* isolates were revealed the high prevalence 9/10 (90%) of *(aad*AI and *qnr*S) genes of tested isolates, in another study *(aad*AI gene) was present in several avian *E. coli* isolates**(Bass *et al*.,1999)** in USA, while in Egypt lower prevalence rate was noted (64%) for (*qnr*S gene) **(Ammar *et al.*,2015)**.

With regarded to the distribution of macrolides and tetracycline resistance genes (*erm*B and *tet*A), the results revealed that 8/10 (80% for each) of *E.coli* isolates, which is higher than the previous studies performed in Egypt (45% for each) **((Ammar *et al.*,2015),** moreover, in our study trimethoprime sulfamethoxazole resistance gene (*sul*I) was detected in (70%) of ten MDR *E. coli* isolates, the results nearly agreed with **(Eid and Erfan ,2013)** who detected (100%) of isolates carried these genes.

For decades, the antibiotic growth promoters has been used to improve poultry performance, while recently the European Union (EU) bans these products and concerns great interest in natural alternatives to over the microbial resistance (**Alcicek *et al*.,2004),**so the current study directed to control colibacillosis by use of herbal medicines. *Neem* (Azadirachta indica) which is one of the most useful traditional medicine.

E.coli cause colisepticemia, coligranuloma (Hjarre's disease), avian cellulites (inflammatory process), air sacculitis, salpingitis, swollen head syndrome, pericarditis, enteritis, peritonitis and panophthalmitis (**Barens and Gross,1997).** In the present study, high mortality rate in infected group (B) (75%) with severe depletion of lymphocytes in spleen and bursa of fabricius, also in some cases, congestion of bursa fabricius and sero-fibrinous exudation in capsule were be observed, moreover, gross lesions of lymphoid organs in group (B) revealed that congestion, enlargement of spleen, atrophied of bursa fabricius, liver and kidney showed congestion and swollen, while in groups (D,E) showed mild congestion, similar signs were recorded by (**Tonu *et al*., 2011).**

The histopathological lesions of lymphoid organs in group (B) showed that spleen sero-fibrinous exudation along with leukocytic cells with infiltration in capsule and depletion of lymphocytes, coagulative necrosis with reticulo endothelial cell hyperplasia, fibrinous exudates are due to acute inflammatory reaction caused by beta hemolycin toxin secreted by *E.coli* which lead to increasing in vascular permeability, this strong injury lead to fibrinogen escaping to the surrounding tissue **(Vagad, 1995)**.

In addition to the previous , bursa of fabricius exhibited severe lymphocytic depletion leading to formation of cystic structure, liver showed degeneration of hepatocytes with vacular degeneration, this pathological changes attributed to detoxification and excretion of endotoxins and enterotoxin appear by *E.coli*, kidney showed tubular degeneration, granular eosinophilic cytoplasm ,necrosis of tubular epithelial cells, and area of hemorrhage in the kidney of broiler chicks, these pathological findings resulted from *E.coli* toxins**,** lungs suffered from severe congestion, infiltration of heterophils, macrophages and lymphocytes in bronchus wall as in the peribronchial alveoli, duodenum revealed severe infiltration of leukocytes mainly lymphocytes, heterophils and macrophages in the submucosa of duodenal wall with enlarged mucosal glands, these pathological findings

attributed to detoxification and excretion of enterotoxin and endotoxins with inflammatory reaction, the results agreed with **(Dutta *et al*., 2012).**

The infected group which treated with *Neem* (NLE) and infected group which treated with amoxicillin groups (D,E), were significant improvement in histopathological lesions. Follicles were observed with reticuloendothelial cells, hyperplasia in spleen and bursa indicating recovery. Clear mild vaculation of sinusoidal spaces, focal areas of infiltrated inflammatory cells and hepatocytes, also mild swollen tubular epithelial cells were observed, these result agreed with **(Kumari and Gupta,2014).** Microscopically, all sacrificed birds in group (D,E) did not reveal homogenous severity of lesions in all organs as study recorded by **(Ghosh *et al.*, 2006).**

On otherwise, the haematological parameters in experimentally *E.coli* infected chicks supplemented with *Neem* leaf extract group (C), revealed a significant decrease in RBCs counts , Hb concentration and PCV% with a significant increase in MCV in chicken in group B lead to macrocytic normochromic anemia due to non-significant change in MCH and MCHC, these anemia may be due to diarrhea or breakdown of erythrocyte by the hemolytic enzymes which produced by *E.coli* , these results agree with **(Justice *et al*.2006** and **Hayder and Shayma,2011)**. Groups (C and D) showed improvement in the hemogram. Also chicks in group (E) were improved more than other group may be due to antioxidant effect of *Neem* and bactericidal effect of amoxicillin on *E.coli* our results agree with **(Sharma *et al*.,2016).**

Concerning the Leukogram, there were a significant leukocytosis, heterophilia and monocytosis in group (B) whereas lymphocyte were significant decrease, these results in the infected group may be due to infection by *E.coli* and inflammation, where heterophils is the first line of defense that lead to leukocytosis, heterophilia and monocytosis which are responsible for phagocytosis of infected microorganism and damaged cells. Alteration of lukogram due to inflammatory reactions caused by infection **(Doxy,1983)**, moreover, lukocytosis is characteristic feature of bacterial infection**,** our result agree with **(Benjamin,2013)**. Groups (C and D) showed improvement of the leukogram when compared with the infected non treated group these results agree with **(Haq *et al.* ,2015).**

Concerning liver enzymes in chicken in group (B) showed a significant increase in serum ALT, AST, these may be due to hepatic damage and release of enzyme from damaged cells into serum, **(Amine *et al*.,2020)** the hepatic damage was confirmed in our histopathological study where liver showed degenerative and necrotic changes in large number of hepatocytes, our result agree with **(Maran *et al*.,2020)** the treatment with amoxicillin with prophylaxis with *Neem* amole rate the liver enzyme toward normal due to high efficiency of amoxicillin in treating infection by *E,coli* with protective effect of neem due to its anti-inflammatory and antioxidant effect . These results agree with **(Patil *et al*,2018).**

Regarding to kidney function test , the uric acid and creatinine were increase in infected group (B), these results may be due to the effect of *E .coli* on kidney lead to degeneration and necrosis of renal tubules which seen histopathologically that prevent their execration leading to increase their levels in serum of infected birds **(Kwawukume *et al.*,2013),** treatment by amoxicillin of infected chicks and prophylaxis by *Neem* improve kidney function by increasing level of creatinine and uric acid toward normal level especially in group (E) which seen histopathologically, our results agree with **(Amine *et al*.,2020)** .

The formation of free radicals or reactive species in cells were suppressed or prevented by the antioxidant. This is very fast in neutralizing any molecules with potential of developing into a free radical. Catalase consider the first line of defense antioxidant, in this study, the infected chicks by *E.coli* showed significant decrease in catalase enzyme this may be due to the oxidative stress produced by *E.coli* infection which lead to increase the use the antioxidant and also on decrease catalase enzyme, these results agree with (**Mehta *et al*., et al.2009)** who found a significant decrease in CAT and SOD in birds infected by *E.coli*, also our study revealed non-significant change in CAT enzyme in group (E) compared with control group (A) (**Ighodaro,2018).**

Chicken infected with *E.coli* (group B)showed a significant increase in immunoglobulin's IgA, IgM when compared with (group A), this is due to the infection with *E.coli* stimulate immune system lead to release of immunoglobulin's as mentioned by **(Kabir *et al*, 2004),** these results were tolerated in group (E) may be due to the immune effect of *Neem* and amoxicillin on *E.coli*, these results agree with (**Jawad *et al*.,2013 and Sharma *et al*.,2016)** who recorded increase production of antibodies against infectious disease by adding *Neem* leaves extract to feed of broiler and enhanced the humeral as well as cellular immune responses attributed to its immune modulatory property.

The results of IL6 showed significant increase in infected groups (B and D) as compared to control group (A), this may be due to inflammation with gram –ve bacteria *E.coli*, same data was recorded by

(**Jae-Won Lee *et al.*,2017)** who stated that gram-negative bacterial sepsis induces high levels of IL-6 in the serum, and IL-6 expression has been suggested as a diagnostic marker of disease state **,** but IL6 in groups (C and E) who treated by *Neem* leaves extract showed non significant increase toward normal level, this results agree with **(Riska and Darmadi, 2020)**,these were due to the inhibition of pro-inflammatory molecules which attributed to the anti-inflammatory effect of *Neem* **(Patil *et al.*,2018**).

5. Conclusion

From this study we concluded that *Neem* leaves extract in water improve the hematological, biochemical and pathological changes that occurred due to experimentally *E.coli* infection in broiler chicken this improvement due to anti-bacterial ,anti-inflammatory and immunomodulator effect of *Neem*.

Data Availability

Samples were submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production for bacterial diseases , sensitivity test for specific antibiotics and PCR confirmation, Animal Health Research Institute, Zagazig laboratory' for hematological and histopathological examination.

Conflicts of Interest

%e authors declare that they have no conflicts of interest.

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