The Pharma Innovation Journal 2022; SP-11(2): 1418-1422

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(2): 1418-1422

www.thepharmajournal.com
Received: 13-12-2021
Accepted: 15-01-2022

Priyanka Tank
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

KK Jakhar
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Vikas Nehra
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Deepika Lather
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Corresponding Author
Priyanka Tank
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Haematological alterations in alpha-cypermethrin intoxicated broiler chicken co-infected with *Escherichia coli*

Priyanka Tank, KK Jakhar, Vikas Nehra and Deepika Lather

Abstract
The poultry industry suffers great losses due to bacterial and viral epidemics and endemics. Amongst these infections, the one caused by *Escherichia coli* (*E. coli*) is quite common and avian pathogenic *E. coli* (APEC) in particular, causes a large number of severe respiratory and systemic pathological conditions/diseases. The ill effects of the infection are found aggravated due to immuno-suppression caused by the pesticides being used for the agriculture purposes. In the present study, impact of alpha-cypermethrin exposure was studied on hematological parameters of poultry experimentally infected with *E. coli*. A total number of one hundred and fifty, day old broiler chicken were taken. Alpha-cypermethrin was given 1/5th of the maximum tolerated dose (i.e. 63.94 mg/kg. body weight) through drops via oral route from seven days onwards till the termination of the experiment. At the age of fourteen, the birds in the treatment group were also experimentally infected with 10^7 CFU of E. *coli* in 1.0 ml normal saline solution via intraperitoneal route. Haematological studies revealed significant reduction in Haemoglobin (Hb), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR), Total Erythrocyte count (TEC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in all the *E. coli* infected and alpha-cypermethrin intoxicated birds as compared to the birds of control group. However, Total leukocyte count (TLC) and Differential leukocyte count (DLC) showed significant increase in *E. coli* infected and alpha-cypermethrin intoxicated group as compared to control group. Thus, alpha-cypermethrin intoxication (1/5th maximum tolerated dose) enhanced the severity of *E. coli* infection as evidenced by haematological findings.

Keywords: Alpha- cypermethrin, *E. coli*, haematological parameters, maximum tolerated dose (MTD)

1. Introduction
Collibacillosis is the most common bacterial disease in poultry caused by *E. coli*. It has worldwide distribution and fatal nature which poses a great threat to economy of the poultry industry. It is an acute systemic disease characterized by multiple organ lesions. A large number of pathological conditions such as pericarditis, perihepatitis air-sacculitis, peritonitis, salpingitis, pan-opthalmitis, omphalitis, cellulitis, colisepticemia, coligranuloma and swollen head syndrome have been observed in case of ollibacillosis [1]. The pesticide participates much to the chemical pollution and its leaching into the environment adversely affects the public health. Pesticide intoxication is also one of the most common causes of occupational hazards in poultry. Among different classes of pesticides, insecticide is an important class which deals with wide range of insects that directly or indirectly harm the cultivated crops. Pyrethroid is the first preference insecticide as it is biodegradable and less toxic to non-targeted species [2]. Cypermethrin is a commercially used synthetic pyrethroid and is widely used to spray the walls of poultry houses or studs/livestock farms against agricultural pests, insects and ectoparasites. Continuous use of these pesticides results into cumulative effect leading to their accumulation in the food chain. These pesticides besides immunosuppression make the animal susceptible to various infections by altering the basic immunological defense machinery of the body. Reckon with the above facts, haematological studies were conducted in the present research work on experimentally *E. coli* infected as well as alpha-cypermethrin intoxicated broiler chicken.

2. Materials and Methods
The study was conducted on a total number of one hundred and fifty, day old broiler chicks after the approval of the Institutional Animal Ethics Committee (VCC/IAEC/20191930-1953).

2.1 Experimental design and sample collection

The present study was undertaken in total one hundred and fifty, day old broiler chicks for evaluating the effects of alpha-cypermethrin toxicity and its interaction with *E. coli* infection on haematological parameters. Maximum tolerated dose (MTD) of alpha-cypermethrin used in the present study for broiler chicken was calculated to be 319.69 mg/kg body weight (b. wt.). At the age of seven days, the chicks were randomly divided into two groups i.e. group A and group B containing 70 and 80 chicks, respectively. All the birds of group B were orally given 1/5th of MTD of alpha-cypermethrin (i. e. 63.94 mg/kg b. wt.) through drops and the group A was kept as control. At the age of 14 days the birds of group B were divided into two subgroups i.e. B_1 and B_2 consisting of 40 birds each. Similarly, group A was also divided into two subgroups i.e. A_1 and A_2 containing 30 and 40 birds, respectively. Thereafter, each birds of subgroups A_2 and B_2 were intraperitoneally injected with 10^7CFU *E. coli* in 1.0 ml normal saline solution (NSS). The experiment was terminated on forty second day. The blood samples were collected directly from heart of six birds from each subgroup on 0, 3rd ,11th, 21st and 28th days post *E. coli* infection in sterile ethylene diamine tetra acetate (EDTA) coated vials for haematological studies.

2.2 Haematological examination

2.2.1 Hemoglobin (Hb) estimation

It was estimated by Cyanmet-Haemoglobin method. The Drabkin's solution (Jupiter Reagent, Techno Biochemic) was used as blank and heamoglobin standard of Central Drug House (P) Ltd. as standard. Place 5.0 ml of Drabkin's diluents solution and add 0.02 ml of blood with Sahli's pipette. Rinse the pipette at least thrice with the diluents. Mix thoroughly and let it stand at least for 10 min. for maximum conversion of hemoglobin to cyanmethemoglobin. Read the OD in the spectrophotometer at 540 nm wavelength.

Concentration of Hb (g/100 ml) = OD of Test/OD of standard X Concentration of Hb in standard

= OD of Test/ 0.453 X 15

2.2.2 Packed cell volume (PCV)

It was estimated by Microhaematocrit method (Cohen, 1967). The microhaematocrit capillary tube was filled with anticoagulant added blood to approximately 3/4th portion through capillary action. The end of the tube containing blood was sealed with clay and then centrifuged in microhaematocrit centrifuge for 5 min at 10,000 to 13,000 rpm. The readings were recorded as percent of PCV by using haematocrit tube reader.

2.2.3 Total erythrocyte count (TEC)

TEC was determined in bright line improved Neubauer Haemocytometer. Natt and Herrik solution was used as diluting fluid for counting total erythrocytes. The diluting pipette used was RBC diluting pipette. The procedure of filling pipette, charging haemocytometer chamber, counting of the cells and calculations were as per the standard protocols.

Total erythrocyte count/µl= X × 10,000

2.2.4 Erythrocyte sedimentation rate (ESR)

The wintrobe haematocrit tube for ESR estimation was filled with anticoagulant containing blood up to 0 mark on the left scale. The tube was set in a vertical position in an appropriate rack. The upper level of sedimenting erythrocyte was read in millimeters on the left scale at 1 h.

2.2.5 Erythrocyte indices

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpscular hemoglobin concentration (MCHC) were calculated employing respective formulae:

$$\text{MCV (fl*)} = \frac{\text{PCV (\%)}}{\text{TEC (million/cu mm)}} \times 10$$

$$\text{MCH (pg**)} = \frac{\text{Hb (g/dl)}}{\text{TEC (million/cu mm)}} \times 10$$

$$\text{MCHC (\%)} = \frac{\text{(Hb (g/dl)}}{\text{PCV (\%)}} \times 100$$

*femto liter, **pico gram

2.2.6 Total leucocyte count (TLC)

The diluting pipette used was RBC diluting pipette and total leucocyte count (TLC) was estimated using Neubauer haemocytometer. Counting of cells in the haemocytometer chamber and calculations were made as per standard methods. Total leucocyte count = X × 500 per cu mm.

2.2.7 Differential leucocyte count (DLC)

The blood smear, prepared from fresh blood was stained with Wright staining method. Air dried smear was covered with Wright's stain (3.3 g of Wright's stain powder in 500 ml of absolute methyl alcohol) and allowed to stand for 8 min. The formalin solution (0.25 ml concentrated formalin in 500 ml distilled water) was added slowly with a dropper, taking caution not to overflow. When the metallic sheen covered the entire surface of fluid, smear was washed off immediately with distilled water. Finally, the smear was differentiated by dipping the slide up and down 6 to 10 times in solution containing ether and absolute methyl alcohol (1:1). Thereafter, the smear was dried and examined under microscope. The counting of different leucocytes was done by battlement method [3].

2.2.8 Statistical analysis

The data for various parameters were subjected to statistical analysis by using Duncan Multiple Range Test as modified by [4] at 5.0 % level of significance using SPSS 16.0 version software. Individual means were compared for statistical significance using least significance difference.

3. Results and Discussion

Mean values of different haematological parameters *viz*. Hb, TEC, PCV, ESR and TLC have been shown in Table 1. A significant ($P \leq 0.05$) decrease in Hb, TEC and PCV of alpha-cypermethrin intoxicated birds of group (B_1) was observed in the present study in broiler chickens. Yousef *et al*. [5] also reported similar findings in rabbits treated with cypermethrin.

In both the *E. coli* infected groups *viz.* A_2 and B_2, there was significant ($P \leq 0.05$) decrease in the values of Hb, TEC and PCV as compared to the birds of control groups (A_2) throughout the experiment. These results were similar as reported by other authors [6, 7, 8, 9, 10]. Hayder and Shayma [11] also observed similar findings in *E. coli* infected male rats. The decrease in haematological parameters may be due to hemolytic enzymes produced by *E. coli* that cause breakdown of erythrocytes [12] or due to inappetence and diarrhoea leading to nutritional deficiencies and this lead to a decrease in a number of erythrocytes which lead to decrease in PCV (%) and haemoglobin concentration [13]. Petrov *et al.* [14] also noticed a decrease in TEC in *E. coli* infection in a weaned rabbit. Mean values of ESR was found to be significantly ($P \leq 0.05$) decreased in alpha-cypermethrin intoxicated group B_1 as well as both the *E. coli* infected groups (A_2 and B_2) when compared to control group (A_1). In general, the ESR increases in anaemia while decreases in liver disease. In the present study, both these conditions were observed which might be responsible for significant change in ESR activity. Mean values of TLC were found significantly ($P \leq 0.05$) decreased in birds of group B_1 as compared to control group (A_1) on 10 DPCT (days post alpha-cypermethrin treatment), 28 DPCT and 35 DPCT. Mean values of TLC were found significantly ($P \leq 0.05$) increased in both the *E. coli* infected groups (A_2 and B_2) as compared to control group (A_1) throughout the experiment.

Table 1: Mean haemoglobin (Hb, g/dl), total erythrocyte count (TEC, 10^6/cu mm), packed cell volume (PCV, %), total erythrocyte count (TEC, 10^6/cu mm) and total leucocyte count (TLC, 10^3/cu mm) values of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.)

Parameter(s)	Groups	Days post *E. coli* infection (DPI)/ Days post alpha- cypermethrin treatment (DPCT)				
		0/7	3/10	11/18	21/28	28/35
Mean haemoglobin (Hb, g/dl)	A_1	8.17^{a}±0.18	8.57^{c}±0.29	9.77^{c}±0.43	11.08^{c}±0.55	10.85^{c}±0.22
	B_1	7.02^{a}±0.32	5.77^{b}±0.18	6.01^{b}±0.26	6.15^{b}±0.28	7.08^{a}±0.33
	A_2	6.80^{a}±0.24	5.93^{b}±0.26	4.28^{a}±0.24	5.47^{ab}±0.22	6.55^{b}±0.19
	B_2	6.90^{a}±0.28	4.48^{a}±0.22	4.03^{a}±0.13	4.85^{a}±0.26	4.93^{a}±0.31
Total erythrocyte count (TEC, 10^6/cu mm)	A_1	3.36^{a}±0.08	3.38^{b}±0.09	4.18^{c}±0.05	3.39^{b}±0.13	3.45^{b}±0.12
	B_1	3.71^{bc}±0.04	3.72^{c}±0.07	4.51^{d}±0.06	3.88^{c}±0.02	3.28^{b}±0.07
	A_2	3.79^{c}±0.05	3.28^{b}±0.08	3.16^{b}±0.03	3.27^{b}±0.12	3.49^{b}±0.02
	B_2	3.63^{b}±0.03	2.78^{a}±0.06	2.67^{a}±0.03	2.86^{a}±0.04	2.99^{a}±0.04
Packed cell volume (PCV, %)	A_1	29.57^{a}±0.48	29.82^{c}±0.56	31.98^{c}±0.62	30.86^{b}±0.40	32.23^{b}±0.87
	B_1	27.32^{a}±0.80	26.78^{b}±0.59	30.08^{c}±0.38	29.82^{b}±0.65	30.62^{b}±0.67
	A_2	26.38^{a}±0.74	23.70^{a}±0.96	17.85^{b}±0.75	22.62^{a}±1.31	26.58^{a}±0.83
	B_2	26.07^{a}±0.78	22.47^{a}±1.24	15.47^{a}±0.87	22.37^{a}±1.25	26.47^{a}±0.76
Erythrocyte sedimentation rate (ESR, mm/hr)	A_1	1.89^{b}±0.22	2.10^{b}±0.17	2.17^{b}±0.13	2.08^{b}±0.09	2.06^{c}±0.08
	B_1	1.86^{a}±0.04	1.73^{a}±0.05	1.59^{a}±0.07	1.57^{a}±0.04	1.40^{a}±0.08
	A_2	1.90^{a}±0.07	1.69^{a}±0.05	1.75^{a}±0.05	1.77^{a}±0.05	1.85^{b}±0.03
	B_2	1.94^{a}±0.08	1.56^{a}±0.11	1.57^{a}±0.07	1.67^{a}±0.07	1.70^{b}±0.06
Total leucocytic count (TLC, 10^3/cu mm)	A_1	19.53^{a}±0.39	28.71^{b}±0.59	29.44^{a}±0.27	29.66^{b}±0.43	27.99^{b}±0.30
	B_1	20.30^{a}±0.19	25.06^{a}±1.33	29.28^{a}±0.28	27.45^{a}±0.24	21.32^{a}±0.31
	A_2	19.79^{a}±0.38	39.86^{c}±0.51	150.39^{b}±0.22	85.48^{c}±0.30	56.69^{c}±0.32
	B_2	22.17^{b}±0.14	41.09^{c}±1.26	162.34^{c}±1.42	100.16^{d}±0.41	62.64^{d}±0.32

a, b, c, d: Means with unlike superscript letters in a column are significantly different, $P \leq 0.05$

Mean values of different erythrocyte indices *viz.* MCV, MCH and MCHC has been shown in table 2. The study of erythrocytic indices revealed that mean values of MCV, MCH and MCHC in group B_1 showed significant ($P \leq 0.05$) decrease from 10 DPCT onwards as compared to control group (A_1). Mean values of MCV, MCH and MCHC also decreased in both the *E. coli* infected groups (A_2 and B_2) when compared to control group (A_1). These results in the present study indicated that the infected and intoxicated birds experienced microcytic hypochromic anaemia. Petrov *et al.* [14] reported a similar decrease in MCV in the offspring of infected mothers as well as in the experimentally infected rabbits. Similar to the present studies, Haq *et al.* [9] also reported a decrease in MCV, MCH and MCHC in *E. coli* infected pigeon. However contrary to the present study, Saini [6] found no significant difference in the mean values of MCV, MCH and MCHC indicating macrocytic normochromic anaemia in *E. coli* infected broiler chicken. A significant increase in MCV and MCH values and no significant difference in MCHC values in broiler chicken due to colibacillosis infection and subsequent NLE supplementation (Neem leaf extract) was reported by Sharma *et al.* [10].

Table 2: Mean corpuscular volume (MCV, fl), Mean corpuscular haemoglobin (MCH, pg) and Mean corpuscular haemoglobin concentration (%) of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.)

Erythrocyte indices	Groups	Days post *E. coli* infection (DPI)/ Days post alpha-cypermethrin treatment (DPCT)				
		0/7	3/10	11/18	21/28	28/35
Mean corpuscular volume (MCV, fl)	A_1	70.17^{b}±2.61	88.45^{b}±1.86	76.59^{c}±1.68	91.76^{b}±4.12	94.34^{b}±5.32
	B_1	73.68^{a}±2.57	72.49^{a}±2.64	66.77^{b}±1.31	76.71^{a}±1.89	93.38^{b}±2.24
	A_2	71.69^{a}±1.71	80.76^{ab}±3.87	58.19^{a}±3.83	78.24^{a}±3.80	88.77^{b}±2.94
	B_2	70.50^{a}±1.90	72.62^{a}±3.47	56.54^{a}±2.51	69.61^{a}±4.79	76.13^{a}±2.73
Mean corpuscular haemoglobin (MCH, pg)	A_1	20.41^{a}±1.15	25.42^{c}±0.94	23.45^{c}±1.32	32.83^{c}±1.76	31.61^{c}±0.92
	B_1	18.94^{a}±0.94	15.59^{a}±0.55	13.34^{a}±0.58	15.79^{a}±0.67	15.85^{a}±1.07
	A_2	18.73^{a}±0.71	21.34^{b}±0.81	16.05^{b}±0.82	19.18^{b}±0.89	21.94^{b}±0.56
	B_2	18.19^{a}±0.91	13.74^{a}±1.04	12.79^{a}±0.56	14.85^{a}±0.77	14.12^{a}±0.87

Mean corpuscular haemoglobin concentration (%)	A_1	27.66^{a}±0.79	28.72^{d}±0.69	30.59^{b}±1.50	35.97^{b}±1.92	33.75^{c}±0.94
	B_1	25.86^{a}±1.49	21.54^{b}±0.59	20.04^{a}±1.06	20.70^{a}±1.17	16.93^{a}±0.85
	A_2	26.26^{a}±1.42	26.51^{c}±0.64	28.56^{b}±3.18	24.94^{a}±2.01	24.90^{b}±1.25
	B_2	26.25^{a}±1.29	18.97^{a}±0.90	22.77^{a}±1.12	21.96^{a}±1.52	18.67^{a}±1.41

a, b, c, d: Means with unlike superscript letters in a column are significantly different, $P \leq 0.05$

Mean values of differential leucocyte count has been shown in Table 3. There was a significant ($P \leq 0.05$) increase in absolute lymphocyte and absolute heterophil count in both the *E. coli* infected groups (A_2 and B_2) as compared to control group (A_1). This indicates leucocytosis due to absolute heterophilia and lymphocytosis. Leucocytosis is mainly encountered in localized or generalized infections, tissue necrosis, acute haemorrhages and acute hemolysis. In the present study, this might be due to *E. coli* infection has led to the necrosis and haemorrhages in the visceral organs. These findings were in accordance with [10] in broiler chicken.

Table 3: Mean differential leucocytes count (DLC, %) of broiler chicks in different experimental groups at a different time intervals (Mean ± S.E.)

Type of Leucocytes	Groups	Days post *E. coli* infection/ Days post alpha-cypermethrin treatment				
		0/7	3/10	14/18	21/28	28/35
Absolute lymphocyte count	A_1	14.08^{ab}±0.55	19.24^{a}±0.52	19.63^{a}±0.41	19.45^{a}±0.57	19.08^{b}±0.29
	B_1	14.08^{ab}±0.40	17.21^{a}±0.94	18.83^{a}±0.29	18.07^{a}±0.31	13.60^{a}±0.25
	A_2	13.89^{a}±0.54	22.16^{b}±0.42	52.89^{b}±1.58	46.16^{c}±0.41	35.44^{c}±0.52
	B_2	15.45^{b}±0.30	22.29^{b}±0.94	53.27^{b}±1.28	43.74^{b}±0.93	37.69^{d}±0.34
Absolute heterophil count	A_1	4.19^{a}±0.11	7.94^{a}±0.40	8.06^{a}±0.42	8.51^{a}±0.34	7.46^{a}±0.38
	B_1	4.58^{ab}±0.32	6.65^{a}±0.33	9.24^{a}±0.48	7.68^{a}±0.37	6.03^{a}±0.37
	A_2	4.44^{ab}±0.14	14.66^{b}±0.86	83.99^{b}±3.89	34.89^{b}±1.35	18.35^{b}±0.99
	B_2	4.95^{b}±0.26	16.45^{b}±0.94	97.07^{c}±4.31	50.59^{c}±1.26	21.31^{c}±1.26
Absolute monocyte count	A_1	0.80^{a}±0.35	0.90^{a}±0.42	1.11^{ab}±0.50	0.93^{a}±0.42	0.89^{a}±0.39
	B_1	0.50^{a}±0.40	0.93^{a}±0.51	1.28^{a}±0.83	0.98^{a}±0.44	1.15^{a}±0.49
	A_2	0.46^{a}±0.43	1.59^{a}±0.89	2.43^{a}±0.66	4.49^{b}±1.42	2.39^{a}±0.83
	B_2	0.72^{a}±0.37	1.97^{a}±0.87	3.01^{a}±1.46	2.58^{ab}±1.00	1.68^{a}±0.72
Absolute eosinophil count	A_1	0.36^{a}±0.08	0.49^{a}±0.20	0.55^{a}±0.25	0.64^{a}±0.19	0.46^{a}±0.18
	B_1	0.54^{a}±0.21	0.52^{a}±0.27	0.49^{a}±.19	0.69^{a}±0.27	0.43^{a}±0.11
	A_2	0.41^{a}±0.21	0.87^{a}±0.27	3.50^{b}±0.92	1.70^{a}±0.85	1.03^{a}±0.47
	B_2	0.44^{a}±0.22	0.48^{a}±0.07	2.99^{b}±0.90	1.33^{a}±0.32	1.14^{a}±0.40
Absolute basophil count	A_1	0.09^{a}±0.04	0.14^{a}±0.06	0.09^{a}±0.06	0.10^{a}±0.06	0.08^{a}±0.06
	B_1	0.04^{a}±0.03	0.08^{a}±.05	0.15^{a}±.06	0.09^{a}±0.06	0.11^{a}±0.05
	A_2	0.03^{a}±0.02	0.18^{a}±0.07	1.00^{b}±0.32	0.14^{a}±0.12	0.19^{a}±0.12
	B_2	0.10^{a}±0.05	0.19^{a}±0.09	1.08^{b}±0.34	0.17^{a}±0.17	0.10^{a}±0.10

a, b, c, d: Means with un like superscript letters in a column are significantly different, $P \leq 0.05$

4. Conclusion

In the present study, attempts were to estimate the concurrent effects of experimentally alpha-cypermethrin intoxicated and *E. coli* infected broiler chicken. Based on 42 day long experimental trial, it can be concluded that alpha cypermethrin potentiates the pathogenic effects of *E. coli* infection as revealed by haemotalogical parameters. There was a significant drop in Hb, PCV, ESR, TEC, MCV, MCH and MCHC along with increase in TLC. A remarkable heterophilia and leucocytocis also indicated towards the severity of infection.

5. Acknowledgement

The authors are thankful to Head of the Department of Veterinary Pathology, LUVAS for support in smooth conduction of the study.

6. References

1. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne, DE. Disease of Poultry. 11th Edn., Iowa State Press, Iowa, 2003.
2. Sharma V. Pathobiological and immunological studies on alpha-cypermethrin toxicity and its interaction with *Salmonella gallinarum* infection in broiler chicken. Ph. D. Thesis submitted to Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, 2018.
3. Benjamin MM. Out line of Veterinary Clinical Pathology. 3rd Edn. The lowa State University press Ames. Iowa, USA, 2013.
4. Krammer CY. Extension of multiple range tests to group correlated adjusted means. Biometrics. 1957;13(1):13-18.
5. Yousef MI, El-Demerdash FM, Kamel KI, Al-Salhen KS. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology. 2003;189(3):223-234.
6. Saini V. Studies on the effect of neem (*Azadirachta indica*) leaf extract on the pathology and pathogenesis of *E. coli* infection in broiler chicks. M.V.Sc. thesis submitted to Chaudhary Charan Singh Haryana Agricultural University, Hisar, 2004.
7. Huff GR, Huff WE, Rath NC, Anthony NB, Nestor KE. Effects of *Escherichia coli* challenge and transport stress on hematology and serum chemistry values of three genetic lines of turkeys. Poultry Science. 2008;87(11):2234-2241.
8. Ahmed MA, Youssef FM, Abdel Rahman AG. Differentiation between *E. coli* strains causing diarrhoea in broiler chicken by using multiplex PCR. The Egyptian Journal of Veterinary Science. 2013;40(1):21-36.
9. Haq KU, Khan AA, Ullah S, Nabi G. Comparative efficacy of Norfloxacin, Clarithromycin and Cefpodoxime against experimentally induced colibacillosis in pigeons. American - Eurasian Journal of Toxicological Science. 2015;7(2):72-82.

10. Sharma V, Jakhar KK, Dahiya S. Immuno-pathological studies on broiler chicken experimentally infected with Escherichia coli and supplemented with neem (*Azadirachta indica*) leaf extract. Veterinary World. 2016;9(7):735-741.
11. Hayder AZ, Shayma ZF. The effect of experimental *Escherichia coli* infection on some blood parameters and histological changes in male rats. Iraqi Journal of Veterinary Medicine. 2011;35(2):22-27.
12. Justice S, Hunstad D, Seed P, Hultgren S. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. Proceedings of the National Academy of Sciences USA. 2006;103(52):19884-19889.
13. Feldman BF, Zinkl JC, Jain NC. Schalms Veterinary hematology. 5th edn. Lippimcott Williams and Wilkins, 2000, 38.
14. Petrov V, Lyutskanov M, Kanakov D. Effects of spontaneous and experimental colibacteriosis on some haematological and blood biochemical parameters in weaned rabbits. Bulgarian Journal of Veterinary Medicine. 2011;14(4):238-246.