Clinicopathological Study of Phytobiotics

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A B S T R A C T

This study was carried out to illustrate the effects of phytobiotics (Orego-stim®, promazine plus®) in prevention of coccidiosis in poultry farms and also to examine these effects on some blood biochemical, haematological and immunological parameters in broiler chickens. One hundred and fifty, one-day old Cobb chicks were used. They were divided into six equal groups, 25 birds each. The 1st group was non-infected non-treated group. The 2nd group was non-infected, treated with promazen- plus (1ml/Liter drinking water/100 birds (0-14 day), 3 ml/Liter drinking water/100 birds (15-28 day) and 5 ml/Liter drinking water /100 birds (29-42 day). The 3rd group was non-infected, treated with orego-stim (0.3 ml/Liter drinking water). The other groups were inoculated intra-crop with $1x10^5$ infective oocysts of field strain of *Eimeria spp*. on the 8th day of age. The 4th group was infected non-treated. The 5th group was promazen- plus (the same dose of group 2). The 6th group treated with orego-stim (the same dose of group 3). Birds received phytobiotics showed better anticoccidial effect, improvement in immunological parameters with good effects on some blood biochemical and haematological parameters were resulted. Phytobiotics can be considered as an ideal growth promoter, highly effective anticoccidial, and immunostimulant agents.

Keywords: *Phytobiotics, Coccidiosis, hematological, biochemical, Immunological, Broilers.*

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1. INTRODUCTION

Coccidiosis is most important intestinal disease in broiler production. It is caused by protozoan parasites of the genus *Eimeria* (Chapman *et al.*, 2010). Due to its multiplication in the intestinal tract, the parasite causes tissue damage (Hafez, 2008). Avian coccidiosis was the most parasitic disease of poultry causing mortality, malabsorption, inefficient feed utilization, impaired growth rate in broilers, and reduced egg production in layers (Lillehoj *et al.*, 2004).

Anticoccidial drugs have been used for the control of coccidiosis over the past 50 years. They can be categorized as polyether ionophorous antibiotics, as lasalocid, monensin, narasin, salinomycin, and semduramicin or synthetic compounds, as chemicals include decoquinate, halofuginone, nicarbazin, robenidine, and zoalene (Chapman, 2001). Most of the chemicals have disappeared from the market and the main reason for this is the rapid selection for resistance in coccidia,

requiring their wise use, moving to another drug before resistance has built up and birds may be given two or more drugs of these chemicals (Naciri *et al.,* 2004). So, naturally occurring compounds are considered the most effective and safe. They can be considered as best substitutes to chemical anticoccidials. Most of the antioxidants occur as dietary constituents and the most commonly investigated natural dietary antioxidant are vitamin E ,vitamin A, zinc, selenium, Plants rich in flavonoids, saponins and tannins and aromatic plants (Masood *et al.,* 2013).

Phytobiotics represent a broad range of bioactive compounds that can be extracted from different plant sources and can modify the gut microflora in broiler chickens (Vidanarachchi *et al.,* 2005). Also, they are plant-derived feed supplements have been demonstrated to improve broiler performance and reduce clinical symptoms associated with coccidial infections (Applegate, 2009). Additionally, plant derived products (Phytobiotics) consider residue-free unlike synthetic antibiotics and safe to be used as the ingredients in the food industry as well as in animal diet as an ideal growth promoter (Li *et al.,* 2016). Additionally, Phytobiotics consist of a broad variety of substances, mainly extracts from plant materials, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root (Burt, 2004).The active molecules include many different secondary plant metabolites, resulting in a broad range of physiological effects like spasmolytic or immune-stimulatory effects (Lee *et al.,* 2003). Various essential oils have many properties in common, such as antibacterial, antifungal, antiviral, antioxidant, or immune-stimulatory properties (Bento *et al.,* 2013).

2. Materials and methods

2.1. Birds:

One hundred and fifty, one-day old apparently healthy Cobb chicks (male and female) were purchased from Al-Watania Poultry Company. The birds were allotted in metal wire-cages. Each group in a separate unit.

2.2. Chemicals and agents used in the experimental protocol:

a- Eimeria acervulina strain:

Pathogenic field strain of Eimeria acervulina was undergoing propagation, purification and sporulation (Animal health research institute, branch Banha) Eimeria was inoculated intracrob in broiler chicken in a dose of $1x10^5$ sporulated oocysts (counted by Mc-Master apparatus) on the 8th day old.

b- Phytobiotics:

Phytobiotics 1 (Promazin plus®); Promazin plus was produced by Tri-one -South korea. It contains Diospyrus lotus 150 gm, Artemisiae capillaris 123 gm, Ascorbic acid 32 gm, and Purified water (USP) up to 1000 ml. It was given at a dose of 1ml/Liter drinking water/100 birds (0-14 day), 3 ml/Liter drinking water/100 birds (15-28 day) and 5 ml/Liter drinking water /100 birds (29-42 day).

Phytobiotics 2 (Orego-stim®); Orego-stim was produced by Meriden Animal Health Co.-United Kingdom. It contains a- pinene, camphene, B-pinene, sabinene, Myrecene, a-phellandrene, a-terpinene, Limonene, 1.8cineole, B-Ocimene, Trpinolene, 1-Octn-3-o 1, trans-Sabinene hydrate, Linalool, Cis-sabinene hydrate, terpinrn-4ol, a-Terpineol, borneol, B-Bisabolene, carvacrol 81.89%, y-terpinrnr 5.1%, p- cymene 3.76% and thymol 2.12%. It was given at a dose of 0.3 ml/Liter drinking water.

2.3. Experimental design:

One hundred and fifty, one-day old chicks were used. The chicks were divided into six equal groups, 25 birds each. The 1st group was non-infected non-treated group. The 2nd group was non-infected, treated with promazen-plus

(1ml/Liter drinking water/100 birds (0-14 day), 3 ml/Liter drinking water/100 birds (15-28 day) and 5 ml/Liter drinking water /100 birds (29-42 day). The 3rd group was non-infected, treated with orego-stim (0.3 ml/Liter drinking water). The other groups were inoculated intra-crop with $1x10^5$ infective oocysts of field strain of *Eimeria* spp. on the 8th day of age. The 4th group was infected non-treated. The 5th group was promazen- plus at the same dose of group 2. The 6th group treated with orego-stim at the same dose of group 3.

2.4. Ration and Water:

The chicks fed starter diet from 1-15 days old and then fed grower diet from 16- 25 days and finally fed on finisher diet from 25 days to marketing age (Steven and John 2008). All diets were formulated to meet the nutrient requirement of the broilers according to recommendations of the National Research Council (NRC, 1994). The feed was sterilized in the oven at 65º C for 18 hours to destroy the probable accidental sporulated oocysts of *Eimeria* which may contaminate the rations. The water was boiled then cooled before offered to the chicks (Seddiek *et al.,* 2008).

2.5. Haematological parameters:

Parameters of hemogram were deterimined according to standarad techniques described by (Jain, 1986) which include RBCs, Hb, PCV, MCV, MCH, MCHC, TLC and differential leukocytic count. Blood films were stained by Giemsa stain. The percentage and absolute value for each type of white cells calculated according to (Feldman *et al.*, 2000).

2.6. Biochemical parameters:

Blood samples were collected from the jugular vein of five chickens per group after 14, 28 and 42 days of inoculation of *E. acervulina* and/or treatment with phytobiotics. The collected samples were allowed to separate the serum and kept at -20ºC for determining the activity of serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) (Reitman and Frankel, 1957). Furthermore, Serum uric acid (Thomas, 1984) and creatinine (Fabiny and Ertingshausen, 1971) were determined.

2.7. Immunological parameters:

Specimens from intestine were collected from all groups after sacrificing and preserved at -80 in freezer after 28 days of inoculated intracrob of *E. acervulina* and/or treatment with phytobiotics, then for PCR examination as follow, IFN-γ (Suzuki *et al.*, 2009), IL10 (Samy *et al.,* 2015).

2.8. Statistical analysis:

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS, 2009) to determine if variables differed between groups, according to (Snedecor and Cochran, 1989). The Shapiro-Willk test was used to test the normal distribution of the data before statistical analysis was performed. Compare between means were conducted by one-way ANOVA and then Duncan's multiple range test (Duncan, 1955).When probability values is less than 5% ($P < 0.05$), we considered there is a statistically significant difference.

3. RESULTS

3.1. Hematological changes:

3.1.1. Erythrogram results:

Hematological analysis on erythrogram parameters in all groups after 14, 28 and 42 days of treatment were summarized in Table (1), revealed that group 2 (phytobiotic 1) and group3 (phytobiotice 2) showed significant ($P > 0.05$) increases in Hb (gm/dl), packed cell volume (PCV %), RBCs (10^6/ml) parameter when compared with group 1(control).On other hand, there were significant decrease in Hb (gm/dl), PCV (%), RBCs (10^6/ml) in group 4 (infected) when compared with group

1(control). Chicks in group 5(infected and treated with phytobiotic 1) and group 6 (infected and treated with phytobiotic 2) showed significant (P > 0.05) increases in Hb (gm/dl), PCV (%), RBCs (10^6/ml) parameter compared with group 4 (infected). Concerning to red blood cell indices there were non-significant changes in group 2 and 3 when compared with group 1.Meanwhile, significant decrease in MCV (fL), MCH (pg) and MCHC (%) in group 4 when compared with group 1. Chicks in group 5 and 6 showed significant (P > 0.05) increases in MCH (pg) and MCHC (%) while, MCV (fL) showed non-significant difference compared with infected group 4 at 14 d. Chicks in group 5 and 6 showed significant (P > 0.05) increases in MCV (fL), MCH (pg) and MCHC (%) compared with group 4 at 28 d. Chicks in group 5 and 6 showed significant (P > 0.05) increases in MCV (fL) and MCH (pg) but, MCHC (%) showed non- significant changes compared with group 4 at 42 d.

3.1.2. Leukogram results:

The data illustrate the changes of leukogram parameters (total leukocytic and differential leukocytic count) in all groups after 14 days of treatment were summarized in Table (2). Group 2 and 3 showed significant (P > 0.05) increase in heterophil but, WBCs, lymphocyte and monocyte showed non-significant changes compared with chicks in group 1. Meanwhile, WBCs, monocyte, lymphocyte and heterophil in group 4 showed significant (P > 0.05) increases when compared with group 1. WBCs, monocyte and lymphocyte showed significant (P < 0.05) decrease while, heterophil showed significant (P > 0.05) increase in group5 and 6 when compared with group 4.

The results of the differential leukocytic count in all groups after 28 and 42 days of treatment were summarized in Table (2). Group 2 and 3 showed significant (P > 0.05) increases in WBCs and heterophil but, lymphocyte and monocyte showed non-significant change compared with group1.There were significant (P > 0.05) increases in WBCs, monocyte, lymphocyte and heterophil in group 4 compared with group 1. On other hand, there are significant (P < 0.05) decreases in WBCs, monocyte and lymphocyte but, heterophil showed that there was significant (P > 0.05) increase in group 5 and 6 compared with group 4.

3.2. Biochemical parameter:

Data demonstrating the changes of biochemical parameters (ALT, AST activities and creatinine, uric acid levels) after 14 days of treatment are summarized in Table (3). The data revealed that there were non-significant changes in biochemical parameters (ALT, AST activities and creatinine ,uric acid levels) in group 2 and 3 when compared with group 1 (control). There were significant increases in ALT, AST activities and creatinine, uric acid level in group 4 when compared with group 1(control). Chicks in group 5 and 6 showed significant decrease in all parameter except in uric acid level showed significant decrease when compared with chicks in group 4.

The result after 28 and 42 days of treatment in all groups were summarized in Table (3) showed that there were non-significant changes in ALT and AST activities also, in creatinine and uric acid levels in group 2 and 3 when compared with group 1. On the other hand, all parameters (ALT, AST activities and creatinine, uric acid levels) showed significant decreases in group 4 when compared with group 1(control). Chicks in group 5 and 6 showed significant decreases in ALT, AST activities, creatinine and uric acid level when compared with group 4.

Our data of intestinal expression in IL-10 level after 28 days of treatment in all groups were summarized in Table (4). There was significant increase in group 2 and 3 when compared with

group 1(control). Also, there was significant increase in group 4 when compared with group 1(control). As well as group 5 and 6 showed significant increase when compared with group 4.While, IL-10 level in group 5 showed significant increase compared with group 6.

Our results showed that intestinal expression in IFN-γ level after 28 days of treatment in all groups were summarized in Table (4). Chicks in group 2 and 3 revealed non-significant difference in IFN-γ level when compared with group 1(control). There was a significant increase in IFN-γ level in group 4 when compared with group 1(control). IFN-γ level in group 5 and 6 showed significant increase when compared with group 4. While, IFN-γ level in group 5 showed significant increase compared with group 6.

Table 1: Erythrogram changes at 14, 28 and 42 days in broilers experimentally infected with *Eimeria acervulina* with or without phytobiotic treatmeant.

	Period	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Hb (gm/dl)	14 d	12.06 ± 0.42^{b}	13.46 ± 0.48^{a}	13.43 ± 0.29^{a}	7.87 ± 0.44^{d}	10.30 ± 0.44^{c}	10.53 ± 0.52^{c}
	28 d	11.9 ± 0.53^{b}	12.92 ± 0.39^{a}	13.00 ± 0.2 ^{a}	8.35 ± 0.25^{d}	10.82 ± 0.11 ^{c}	10.47 ± 0.21 ^{c}
	42 d	11.50 ± 0.28 ^{b}	12.86 ± 0.40^{a}	12.56 ±0.24^{a}	9.64 ± 0.12 ^{d}	10.86 ± 0.18^{c}	10.58 ± 0.19^{c}
RBCs (10^{6}/ml)	14 d	3.53±0.22^{b}	4.06 ± 0.24^{a}	4.00 ± 0.11^{a}	2.83 ± 0.08^{d}	3.42 ± 0.15^{bc}	3.36 ± 0.08^{bc}
	28 d	3.65 ± 0.11^{b}	4.12 ± 0.4 ^{a}	3.56±0.28^{c}	6.38±0.37 ^{a}	4.52±0.38^{b}	4.77±0.48^{b}
	42 d	3.22±0.18^{c}	3.31±0.25^{c}	3.35±0.19^{c}	5.44±0.38 ^{a}	3.98±0.27 ^{b}	4.11±0.22 ^{b}
PCV (%)	14 d	34.66 ± 1.66 ^{b}	39.00 ±0.57^{a}	38.66 ± 0.66^{a}	26.00 ± 0.57^{d}	30.33 ± 0.33^{c}	30.33 ± 0.33^{c}
	28 d	35.22± 0.71 ^{b}	37.32 ± 0.57^{a}	37.22 ± 0.42^{a}	26.5 ± 0.64^{d}	31.25± 0.75^{c}	31.50 ± 0.64 ^{c}
	42 d	30.50 ± 1.85^{b}	34.00 ± 2.15^{a}	33.70 ± 1.85^{a}	26.05 ± 0.73 ^{c}	30.41 ± 2.31^{b}	29.61 ± 0.40^{b}
MCV (fl)	14 d	98.28 ± 2.58^{a}	96.48 ± 4.92^{a}	96.74 ± 1.62^{a}	89.48 ± 0.91^{bc}	91.81 ± 2.96 ^{b}	90.27 ± 3.38 ^{b}
	28 d	96.03±1.67^{a}	95.54±2.84^{a}	95.18±3.81^{a}	87.33±4.30 ^{c}	91.62±3.57 ^{b}	91.38±0.93 ^{b}
	42 d	96.05±4.36^{a}	96.58±3.81^{a}	97.22±2.23^{a}	88.34±1.32^{c}	90.82±2.26^{b}	91.12±3.46^{b}
MCH (pg)	14 d	34.33±0.73^{a}	33.21±0.90 ^{a}	33.66±1.49 ^{a}	27.65±1.37 ^{c}	30.60±2.46^{b}	31.25±0.94^{b}
	28 d	32.67±1.57 ^{a}	33.17±1.10 ^{a}	33.19±1.54 ^{a}	26.49±1.26 ^{b}	31.81±1.66 ^{a}	30.60±1.52 ^{a}
	42 d	30.02±0.81 ^{a}	33.05±0.96 ^{a}	33.52±0.54^{a}	26.12±0.87^{b}	31.07±0.5 ^{a}	31.04±0.73 ^{a}
MCHC (%)	14 d	35.22±0.57 ^{a}	34.51±0.83^{a}	34.78±1.33^{a}	28.10±1.37 ^{b}	32.11±1.79^{a}	33.77±2.08^{a}
	28 d	34.22±1.34 ^{a}	34.91±0.26 ^{a}	35.15±0.81 ^{a}	29.50±0.39 ^{b}	33.59±0.77 ^{a}	33.33±1.33 ^{a}
	42 d	34.89±0.24^{a}	35.75±1.83^{a}	35.04±0.26^{a}	29.37±0.76 ^{c}	32.41±0.90^{bc}	31.86±0.81^{bc}

Results were expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $p < 0.05$.

Group1: Control Group 2: phytobiotic 1 Group 3: phytobiotic 2 Group 4: infected with coccidia Group 5: phytobiotic 1 infected with coccidia Group 6: phytobiotic 2 infected with coccidia.

Table 2: Leukogram parameters at 14, 28 and 42 days in broilers experimentally infected with *E. acervulina* with or without phytobiotic treatmeant.

	Period (day)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
WBCs	14 d	23.54±0.81^{cd}	25.17±0.32^{c}	25.55±0.21^{c}	48.42±0.22 ^{a}	40.54±0.41^{b}	41.52±0.3 ^{b}
	28 d	24.33± 0.82 ^{d}	28.12±0.71^{c}	29.90±0.62^{c}	53.42±0.74^{a}	44.27±0.64^{b}	45.14±0.71^{b}
(10^3/µl)	42 d	25.85±0.85^{d}	29.64±0.75^{c}	29.16±0.65^{c}	47.65±1.21^{a}	40.35±1.13^{b}	41.60±1.22^{b}
Heterophil	14 d	5.75 ± 0.67^{d}	7.66 ± 1.45^{c}	7.89 ± 1.85^{c}	18.56±2.24^{b}	23.20± 1.54^{a}	21.86±2.33^{a}
	28 d	8.22±0.62^{d}	11.50±0.53^{c}	11.22±0.42^{c}	20.11±0.81^{b}	24.26± 0.65^{a}	23.91±0.43^{a}
(10^3/µl)	42 d	8.44± 0.64^{d}	10.88±0.56^{c}	10.50±0.44^{c}	18.82 ± 1.22^{b}	22.23 ± 0.84^{a}	22.54 ±0.77^{a}
Lymphocyte	14 d	17.71±1.66^{b}	17.42±1.66^{b}	17.55±1.60^{b}	28.21±1.45^{a}	17.48±1.42^{b}	18.78±1.51^{b}
	28 d	17.66±0.58^{b}	16.06±0.47^{b}	16.14±0.81^{b}	31.30±1.90^{a}	18.51±0.42^{b}	19.78±0.61^{b}
(10^3/µl)	42 d	18.17±1.06^{b}	16.44±0.63^{b}	16.33±1.02^{b}	29.48±1.09^{a}	17.24±1.02^{b}	18.21 ±1.03^{b}
Monocyte	14 d	0.08 ± 0.03^{c}	0.09 ± 0.05^{c}	0.11 ± 0.03^{c}	1.62 ± 0.03^{a}	0.86± 0.06^{b}	0.88 ± 0.05^{b}
	28 d	0.45± 0.03^{c}	0.53± 0.04^{c}	0.54± 0.05^{c}	2.01 ± 0.11^{a}	1.50± 0.11^{b}	1.45 ± 0.12^{b}
(10^3/µl)	42 d	0.22±0.03^{c}	0.32± 0.04^{c}	0.33± 0.03^{c}	1.35± 0.06^{a}	0.88± 0.08^{b}	0.85± 0.07^{b}

Results were expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicates significant differences at $p < 0.05$.

Table 3: Serum biochemical parameters at 14, 28 and 42 days in broilers experimentally infected with *E. acervulina* with or without phytobiotic treatmeant.

	Period (day)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Creatinine	14 d	0.26 ±0.01^{c} 0.27	0.24 ±0.01^{c}	0.26 ±0.01^{c}	0.37 ± 0.01^{a}	0.30 ±0.01^{b}	0.30 ±0.01^{b}
	28 d	±0.01^{c} 0.26	0.26 ±0.01^{c}	0.28 ±0.01^{c}	0.46 ±0.01^{a}	0.36 ±0.01^{b}	0.34±0.03^{b} 0.33
(mg/dl)	42 d	±0.01^{c}	0.28 ±0.01^{c}	0.27 ± 0.01^{c}	0.38 ± 0.01^{a}	0.32 ± 0.02^{b}	± 0.01^{b}
Uric acid	14 d	3.49±0.23^{b}	3.45±0.15^{b}3.	3.50±0.25^{b}3.	5.39±0.42 ^{a}	5.26±0.38^{a}	4.95±0.30^{a}
	28d	3.54±0.21^{c}	70±0.24^{c}3.31	56±0.28^{c}3.35	6.38±0.37 ^{a}	4.52±0.38^{b}	4.77±0.48^{b}
(mg/dl)	42 d	3.22±0.18^{c}	±0.25^{c}	±0.19^{c}	5.44±0.38 ^{a}	3.98±0.27 ^{b}	4.11±0.22 ^{b}
ALT	14 d	24.75±2.88^{c}	22.75±2.41^{c}	23.52±2.88^{c}	50.21±3.77^{a}	40.31±2.88^{b}	41.22 ±1.22^{b}
	28 d	25.65±2.21^{c}	25.50±2.39^{c}	26.50±2.78^{c}2	60.25 ±5.72^{a}	48.50 ±5.54^{b}	47.00 ±7.76^{b}
(U/L)	42 d	27.20±1.99^{b}	26.65±6.21^{c}	5.71±5.52^{b}	55.84 ± 8.23^{a}	43.77 ±7.99^{b}	44.37± 8.74^{b}
AST	14 d	175.33 ± 4.41^{c}	176.33±5.11^{c}	170.66±4.40^{c}	220.32 ±7.69^{a}	194.33± 5.52^{bc}	189.66 ±5.88^{bc}
	28d	178.50 ±5.48^{c}	180.55±4.85^{c}	177.25±4.55^{c}	230.84 ± 8.65^{a}	199.44 ± 6.2 ^{bc}	95.72 ± 7.35 ^{bc}
(U/L)	42 d	179.66 ±5.52^{c}	178.65±5.44^{c}	175.22±4.55^{c}	260.57 ± 8.56^{a}	235.22±6.33^{b}	231.15 ±6.61^{b}

Results were expressed as mean ±S.E.M. Different superscripts (a, b and c) within the same column indicate significant differences at $p < 0.05$.

Table 4: Relative gene expression (IL-10 and IFN-γ) in the intestine in broilers experimentally infected with *E. acervulina* with or without phytobiotic treatmeant.

Groups	IL-10	IFN-γ
Group 1	1.00 ± 0.00 ^{e}	1.00 ± 0.00 ^{e}
Group 2	1.86 ± 0.15 ^{d}	1.43 ± 0.16 ^{e}
Group 3	1.75 ± 0.13^{d}	1.30 ± 0.18 ^{e}
Group 4	4.64 ± 0.23 ^{c}	5.91 ± 0.67 ^{c}
Group 5	8.56 ± 0.35 ^{a}	9.22 ± 0.56 ^{a}
Group 6	6.67 ± 0.26 ^{b}	7.21 ± 0.75 ^{b}

Results were expressed as mean ±S.E.M. Different superscripts (a, b, c, d and e) within the same column indicate significant differences at $p < 0.05$.

4. DISCUSSION

Concerning to Haematological Parameter, our data revealed that infection with *E. acervulina* lead to decrease PCV, RBC_S and Hb. These data in accordance with (Akhtar *et al.*, 2015).The reduction in Hb and total erythrocytic count may be due to haemorrhages in the caeca followed by development of caecal lesions (Hein, 1971).The decline in the rates of PCV in an infected group, due to anemia as a result of infection with *E. tenella* due to penetration of the parasite to epithelial cells leading to bleeding and thus decrease the number of RBCs and PCV (Mohammad *et al.*, 2016). On the other hand these data disagree with Adenaike *et al.*, (2016) who showed that there were no significant differences in haematological values. This may be due to different species of *Eimeria.*

In this study, the data revealed that there were significant increases in hematological parameters (Hb, PCV and RBCs) between groups treated with phytobiotics and non-infected with *Eimeria* when compared with control group. Also, groups treated with phytobiotics and infected with *Eimeria* showed significant increases in hematological parameters (Hb, PCV and RBCs) when compared with infected group. These results agree with Mohammad *et al.*, (2016) who mentioned that The effect of Artemisia herba alba (ingredient of phytobiotic 1) lead to increase the PCV and due to the containing of these herba high salts such as sodium, magnesium, calcium, potassium, iron and zinc, which has some of the properties to stimulate the production of RBCs, the fact that Artemisia herba alba reduces the level of oxidized red blood cells membranes and thus reduce the decomposition of these cells; therefore increase the number and their Hb concentration. Most of the properties of plants are due to the essential oils (EOs) and other secondary plant metabolites. Eos as thymol, carvacrol, eugenol (ingredient of phytobiotic 2) enhances production of digestive secretions and stimulates blood circulation (Brenes and Roura, 2010).

Concerning to leukogram, our data revealed that there were leukocytosis, heterophilia, lymphocytosis and monocytosis in groups infected with *E. acervulina* when compared with non-infected groups. Our data agree with (Amad *et al.*, 2013; Akhtar *et al.*, 2015). The increased WBCs and heterophil counts might be attributed to the induction of an immune response in the infected birds because these cells participate as the first line of defense

against many infections (Campbell and Ellis, 2007).

Our data showed that there were improvement in WBCs, monocyte and lymphocyte toward control group. While, heterophil showed significant increase in groups treated with phytobiotics compared with non-treated groups. These due to role of phytobiotics as anti-coocidial (Allen *et al.,* 1997) and anti-inflammatory effect (Xie *et al.,* 2008). This is supported by our findings of increased IL-10 in the presence of phytobiotics treatment. The increase in the heterophils was observed because heterophils contain a variety of granules that contribute to the first line host defense against bacteria, fungi, protozoa and some viruses. Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds (Irizaary–Rovira, 2004).

In regard to biochemical parameter, in this experiment (AST, ALT activities) showed a significant elevation in serum activities of these enzymes in infected group with *Eimeria acervulina* when compared with non-infected group. Similar out comes have been observed by (Patra *et al.,* 2010; Dar *et al.,* 2014) and can explained by them as increase in the level of serum enzymes might be due to cellular damage particularly of hepatocytes.

On the other hand, our results disagree with Adamu *et al.* (2013) reported that there were decreases in ALT and AST activity when 10 prominently ill chickens collected from infected broiler flock with *E. tenella* and *E. brunetti* on 5th week of age. This disagreement is due to different doses and species of *Eimeria*.

The results of present work reported that treatment with phytobiotics in groups 5 and 6 induced significant decreases in serum AST and ALT when compared with group (4) infected with *E. acervulina* without treatment. These results were in accordance with (Youssef *et al.,* 2013).The effect of phytobiotics may be due to anti-inflammatory effect (Hashemi and Davoodi 2011; Wang *et al.,* 2015).

Concerning to kidney function, creatinine and uric acid levels in infected group with *E. acervulina* (4) showed significant elevation in serum level when compared with non-infected group 1(control). These results agreed with (Patra *et al.,* 2010; Dar *et al.,* 2014). The significantly higher serum uric acid and creatinine level in infected birds may be due to severe kidney dysfunction, metabolic acidosis, as well as intravascular haemolysis (Patra *et al.,* 2010). Also, Serum ALT and AST activities, uric acid and creatinine were significantly increased in infected groups of chickens this may be due to the impaired liver function and injury of liver and kidney parenchyma due to the harmful effect of *Eimeria* parasite (Youssef *et al.,* 2013).

Our result showed that treatment with phytobiotics induced significant decreases in serum uric acid and creatinine when compared with groups infected with *E. acervulina* without treatment. These results agreed with Youssef *et al.,* 2013; Tollba, 2010). These results were explained as Origanum vulgare used in the treatment of urolithiasis prevented as well as reversed toxic changes containing loss of body weight, polyurea, crystalluria, oxaluria, raised serum urea and creatinine levels and crystal deposition in kidneys (Khan *et al.,* 2011). Also, due to its anti-inflammatory effect and anti-oxidant effect (Rimini *et al.,* 2014). Moreover, the anti-oxidant effect enhanced liver and kidney functions (Hermandez *et al.,* 2004).

Concerning to immunological and inflammatory parameter, IFN-γ, the result showed that there was significant increase in intestinal expression in IFN-γ in infected

groups when compared with non-infected groups. These results in accordance with (Dalloul and Lillehoj 2005; Hong *et al.,* 2006). IFN-γ is a major factor in the development of resistance to *Eimeria*, as it inhibits its development. Also, IFN-γ demonstrated significant reductions in intracellular sporozoite development without affecting sporozoite invasion of host cells and decreased oocyst production and significant improvement in body weight gain following *Eimeria acervulina* challenge infection (Lillehoj and Choi, 1998). Our result showed that there were significant increases in IFN-γ in the groups that treated with phytobiotics. These results agreed with (Wang et al., 2016; Schepetkin and Quinn, 2006).This high production of IFN-γ may contribute to clearance of the infection and the development of immunity to reinfection. IFN-γ induces iNOS expression in several cells types, including epithelial cells (Witthoft *et al.*, 1998).

IL-10 is an anti-inflammatory cytokine that controls the nature and extent of inflammatory responses during infection with viruses, bacteria, fungi, protozoa and helminthes (Couper *et al.,* 2008). The result showed that there was significant increase in infected groups when compare with non-infected groups. This result in accordance with (Dalloul and Lillehoj, 2005; Hong *et al.,* 2006). Our result demonstrating that groups that treated with phytobiotics showed significant increase. This result agreed with (Rothwell *et al.,* 2004; Lu *et al.,* 2014). The result showed that phytobiotics is characterized by highly production of IL-10 due to its anti-inflammatory effect.

5. Conclusion

phytobiotics can be used as a potential alternative anticoccidial in broilers to avoid side effects of chemical antibiotic drugs such as antibiotic residue and resistance. Phytobiotics has good effect on heamatology and biochemical parameter. Moreover, phytobiotics has immune stimulatory effect by enhancing immunity and anti-inflammatory effect.

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