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Comparative biochemical and pathological studies between Toltrazuril and garlic supplementation in chickens experimentally infected with coccidiosis *Shahira H.M. Hussein, **Soad Mekawy,***Mervat A. Ayoub ,***Gehan N.A. and ****Rania A.A.

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ABSTRACT

Coccidiosis is one of the significant problems in the poultry industry throughout the world. It is responsible for 6–10% of all broiler mortality. The present study was conducted to evaluate the effect of combination between garlic powder and half therapeutic dose of Toltrazuril for controlling clinical cecal-coccidiosis, based on clinical signs, mortality rate, oocysts counting, hematological, biochemical parameters of blood and pathological examination. One hundred at one day-old chicks were randomly divided into 5 equal groups. The 1st group (A) was kept uninfected (control negative group). The next B, C, D and E groups were infected with 20,000 sporulated oocysts per chicken at the 20th days-old. Group (B) was infected but non treated (control positive). Group (C) was treated with 7 mg/kg Toltrazuril 3rd day P.I. which is the commercial therapeutic dose. Group (D) was fed on ration containing garlic powder 8 gm /kg ration as a prophylaxis. Group (E) was administered garlic powder 8gm/kg ration from the 1st day age then given 3.5 mg/kg toltrazuril (half the therapeutic dose) at the 3rd day post infection (P.I.). In general the clinical signs were huddling, severe depression, chilling, reduction in feed intake, then profuse bloody dropping which decrease gradually by the 11th day P.I. The mortalities were recorded in groups (B, C and D) (35%, 10% and 20% respectively). The hemato-biochemical parameters were significantly decreased in Hb, RBCs, PCV, serum protein level and significantly increased in TLC, AST and ALT levels. The pathological findings showed severe hemorrhagic enteritis, severe leucocytic infiltration and necrotic cecal glands. The birds in group (E) showed milder symptoms with improvement of clinical signs, averages feed intake, as well as hematological, biochemical parameters and pathological lesions with decreasing the oocyst number than in-group (B).

It could be concluded that the combination between garlic powder as prophylaxis and half dose of Toltrazuril as treatment prevent the mortalities with reduction of the number of *Eimeria* oocysts and improve hematobiochemical and pathological results in the affected birds

Introduction

Intestinal coccidiosis is a large group of parasites infecting the gut induced by contaminated food with oocysts. *Eimeria* spp. are belong-

ing to the phylum *Apicomplexa* causing coccidiosis of farm animals and birds. *Eimeria tenella* is confined to ceca of chickens where it causes cecal coccidiosis and cecal hemorrhages

with high mortalities (Shirley 2000). Cecal-coccidiosis in chicken commonly caused by *E.tenella* is usually in young chicks and *E.necatrix* which developed in small intestine (early stages) then later in the cecum (sexual stage). The economic significance of coccidiosis is attributed to decreased production (higher feed conversion, growth depression and increased mortality) and the costs involved in treatment and prevention (Peek and Landman 2011). Badrawy (2012) studied the efficacy of anticoccidial drugs in controlling coccidiosis experimentally in broiler chickens. Their study pointed out that toltrazuril treated chicks showed great improvement in feed intake, average weight gain and food conversion rate, as well as high reduction in the number of oocysts. Rashid et al. (2009) administered toltrazuril orally in drinking water, different doses of toltrazuril (1.75, 3.5 and 7.0 mg) on day 18 and 19 and 25. The result indicated that the lower doses (3.5 mg/kg and 1.75 mg/kg) of toltrazuril proved far better than the higher dose in the weight gain and reduction of oocyst per gram (OPG) counts. Mathis et al. (2004) referred that the use of toltrazuril as the anticoccidial in the drinking water between 10 and 14 days post infection at the rate of 7.0 mg/kg body weight would be the best time for good coccidiosis control, all treatments but days 2-3 allowing full performance because it is broad spectrum against coccidian which acts in the early schizogony. Lakkundi et al. (2002) evaluate the anticoccidial effect of toltrazuril that degenerate the first generation of schizonts and gametes. European Medicinal Agency (1998) reported that toltrazuril inhibit the enzymes of the respiratory chain of the parasite which cause obstruction of wall forming bodies of Eimerian macrogametes. The drug has maternotoxicity and embryotoxicity in dose more than 3 mg/kg bw/day and induce high abortion level in pregnant rabbits. Majed et al. (2019) reported that the highest capacity of crypt and villi of small intestines by the addition of garlic in the broilers feed infected with coccidiosis. The results obtained in the treated groups with amprolium and garlic powders damage the *Eimeria* cells. The lower mortality (higher survival rate) in supplemented groups may be due to Allicin in garlic, which causes oxidative stress to parasites and neutralize oxygen reac-

tive species. Showkat et al. (2014) study was conducted to evaluate the effect of garlic extract on coccidial induced hemato-biochemical alterations in broiler chicken. Garlic treated group birds showed significant increase in Hb, PCV, MCHC and significant decrease in TLC values than in infected group birds from 7 day post infection. The biochemical harmful effects of *Eimeria tenella* on liver and kidney function tests were improved in groups feed on garlic 8.0 mg/kg rations feed additives. El-Khtam et al. (2014) reported the clinical signs, lesion score and oocysts number decreased in garlic supplemented groups compared with untreated groups.

The aim of this study:

1. To evaluate the effect of therapeutic dose of toltrazuril on cecal coccidiosis.
2. To study the anticoccidial effect of garlic powder.
3. To compare the hematological, biochemical and pathological changes after administering of half therapeutic dose of toltrazuril in combination with garlic powder in chickens infected with cecal coccidiosis.

Material and methods:

Chicks: One hundred, at one day old Hubbard mixed breed broiler chicks were obtained from a private poultry farm (Cairo Company of chickens) and were kept under hygienic conditions. Experimental diets (anticoccidial free commercial broiler feed). Vaccination program was done according to Giambrone and Ronald (1986).

Oocysts of cecal Eimeria: Cecal *Eimeria* oocysts for experimental infection were identified according to Brown et al. (1982). The suspected samples were emulsified in water and pass through double layer of gauze to remove debris. The oocysts were separated by using floatation techniques (Soulsby 1982). The dropping solution was mixed with 10 ml saturated salt solution and centrifuged for 5 minutes at 1500 r.p.m. The floating oocysts of the fluid were collected. The collected oocysts were kept at room temperature in 2.5% potassium dichromate solution and spread into thin layer in petri dishes till sporulation. The sporulated oocysts were cleared and counted per 1.0 ml of the solution using the mc Master slide as

described by Long et al. (1976). The collected sporulated oocysts of cecal *Eimeria* were counted and used for induction of experimental infection orally by gastric tube at 20th day-old chickens.

Drug: Toltrazuril: Toltrazuril solution (Deltazuril 2.5%, Delta Pharma Company) was administered at the therapeutic and half therapeutic doses (7 mg and 3.5mg /kg b.wt) in group C and E respectively in drinking water for two successive days at the third day post infection.

Collection and preparation of garlic (*Allium sativum*) The garlic powder were purchased from the local commercial market. It was administered to the chicks from the 1st day old daily to the end of the experiment by dose of 8.0 gm /kg ration for groups D and E (Pourali et al. 2014)

Experimental design

A total of 100 birds were divided into five equal groups (A, B, C, D and E) each consisting of 20 birds.

Group A: was considered as control negative, non-infected non-treated.

Group B: was infected with 20,000 sporulated *Eimeria oocysts* orally by gastric tube on the 20th day old considered as positive control and sacrificed one and two weeks post infection (P.I.).

Group C : was infected with the same dose of sporulated *Eimeria oocysts* on 20th day old chicks and treated with toltrazuril (7.0 mg / kg b.wt.) in drinking water at 3rd day P.I. for 2 successive days and sacrificed one and two weeks post infection .

Group D: was administered garlic dried powder in ration from one day old chicks till the end of experiment (8.0 gm / kg ration). The chickens infected with the same dose of sporulated oocytes by gastric tube at the 20th day old and sacrificed after one and two weeks post infection.

Group E: was administered garlic powder from the 1st day of age as 8.0gm/kg ration and was infected with the same dose of sporulated *Eimeria oocysts* by gastric tube at the 20th day old chickens. Then administered half dose of toltrazuril (3.5 mg/kg b.wt.) in drinking water for 2 successive days and sacrificed one and two weeks post infection.

Evaluation of the treatment efficacy was done according to

1. Clinical symptoms and mortality rate.

2. Bodyweight and weight gain.

3.Oocysts count and treatment efficacy: The output of *Eimeria spp.*in the dropping of infected chickens was counted one and two weeks P.I. by using McMaster chamber method (Long et al. 1976).

$$\frac{\text{Number of oocytes} / \text{gram} = \text{total No.of oocysts in 1st chamber} + \text{total No.of oocysts in 2nd chamber} \times 100}{2}$$

$$\text{Efficacy\%} = \frac{\text{number of oocysts in positive control} - \text{number of oocysts in treated}}{\text{number of oocysts in positive control}}$$

4. Hematological investigation: Blood samples were collected from randomly selected 5 birds of each group at 1st and 2nd week post inoculation. The blood for hematological studies was collected in vials containing disodium salt of ethylene diaminetetra-acetic acid (EDTA) as an anticoagulant. The hemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were done as per standard methods described by Schalm et al. (1975).

5. Serum biochemical examination: Blood samples were collected from chicks after one and two weeks post infection from all groups by puncture of the wing vein into clean dry tubes without anticoagulant to obtain serum. The serum was separated for determination of alanine amino transferase (ALT) and aspartate amino transferase (AST), (Reitman and Frankel. 1957) creatinine, (Slot 1965) uric acid (Sanders and Pasman 1980) total protein, albumin and globulins (Doumas et al. 1980).

6. Post mortem finding and lesion score: Number of positive control lesions scoring was conducted in 5 birds from each group at one and two weeks post infection and scoring scale was graded from 0 to 4 according to Johnson and Reid (1970).

Grade (1):-Light redness of the cecal wall Mild thickening of the cecal intestinal wall. Focal lesions in 3.0 cm diameter of the cecal wall.

Grade (2):- Moderate redness of the cecal wall Moderate thickening of the ceca lwall. Focal lesions in 3.0 cm diameter of the cecal wall. Ballooning in the caecum with normal cecal content.

Grade (3):- Bleeding was more severe with little fecal contents Severe congestion of the cecal wall. Increase the thickening of the cecal wall. Ballooning in the cecum and presence of bloody cecal core.

Grade (4):- Severe bleeding, more thickened cecal wall and erosion of the serosal surface . Death may occur suddenly at 5th day P.I.

7. Histopathological examination: Specimens were collected from cecum, liver and kidneys of dead and sacrificed chicken then were fixed in 10% neutral buffered formalin, then processed to obtained 4 μ m paraffin sections and stained with Hematoxiline and Eosin (H&E) and prepared for microscopical examinations (**Bancroft and Stevens 1990**)

8. Statistical analysis: The generated data were analyzed by one way ANOVA as described by **Tamhan and Dunlop (2000)**.

Results

1-Body weight and weight gain:

In table (1),The chickens in groups(D and E) showed significant increase of body weight during the rearing time before the experimental infection than the control group (A). Chickens in groups (C) and (D) (treated with toltrazuril and garlic respectively) showed significant increase in body weight and weight gain than those in control positive group (B) in both first and second weeks P.I.

There is no significant difference between body weight of chickens in both groups (C) and (D) in the 1st and 2nd weeks P.I. Chickens in group (E) treated with both garlic and half dose of toltrazuril showed more significant increase in body weight and weight gain compared with those in control positive group (B) and both groups (C) and (D) in the 1st and 2nd weeks P.I. So, chickens treated against coccidiosis with garlic together with half dose of toltrazuril group (E) showed better significant effect in body weight and weight gain than those treated

with full dose of toltrazuril (group C) or garlic only (group D) especially in the second week P.I.

2-Clinical signs and mortality rate:

In table (2), chickens in the infected group B (control +ve) showed symptoms include severe bloody diarrhea, ruffled feathers, huddling, weight loss, depression, paleness, lack of appetite, poor weight gain and the area around the vent stained with blood. Seven chickens of all twenty were died at the first week P.I. (35% mortalities). The chickens treated with toltrazuril group (C) showed less severe symptoms, most chickens showed creamy caseated reddish diarrhea and two chickens were died during the first week P.I (10% mortalities). In group (D) (treated with garlic) 4 chickens were died in the first week P.I. (20% mortalities) and they appeared with pale combs, depression and poor weight gain. While the chickens of group (E) (treated with half dose of toltrazuril and garlic) showed milder symptoms. All the treated chickens in group (E) showed gradual improvements of their condition few hours after treatment and no mortalities were recorded. At the second week post infection, symptoms improved in all groups except group (B). No mortality was recorded at the second week P.I.

3-Oocysts counting (OPG), lesion score and treatment efficacy:

One week P.I. there were no significant differences of OPG count in chickens of both groups (B) (control +ve) (62413 ± 1680) and group (D) (administered garlic as prophylactic) (41861 ± 651). While there was a significant difference in OPG count in chickens of both groups © (treated with toltrazuril) (15749 ± 692) and (E) (treated with garlic and half dose of toltrazuril) (15180 ± 780) compared with the control positive group (B)(62413 ± 1680). At the end of second week P.I., the OPG in the chickens of group (D) (5340 ± 430) showed significant difference than control positive group (B)(62413 ± 1680). On the other hand, there is better significant difference in OPG in both groups (C) (991 ± 244) and (E) (705 ± 131) compared with the control positive group (B)(18257 ± 1291). The lesion score in the first week P.I. of groups B, C, D and E were 3.5, 2.5, 3.0 and 2.2 respectively. While the lesion score were 1.9, 0.9, 1.6 and 0.9 respectively in the Second week P.I. Treatment effi-

cacy of the chicken group (E) (administrated half dose of toltrazuril and garlic) revealed better results 75.6% than both groups (C) (toltrazuril) 74.9% and group (D) (garlic) 32.76%. (Table 2)

Table (1) The response of body weight and weight gain in chicken groups (n=5)

Groups	Before infection at 20 day old	First week P.I.		Second week P.I.	
	Bodyweight	Body weight	Weight gain	Body weight	Weight gain
Group A (Control negative)	654±8.69 ^b	1005.60±4.55 ^a	350.80±11.31 ^a	1406.40±3.80 ^a	400.80±4.70 ^a
Group B (control positive)	656±6.92 ^b	780.00±7.07 ^d	124.00±9.61 ^c	1053.40±12.40 ^c	253.40±8.61 ^c
Group C (toltrazuril)	665±4.23 ^b	860.00±7.09 ^c	195.00±7.85 ^b	1194.60±7.49 ^b	334.60±11.4 ^b
Group D (garlic)	763±5.64 ^a	894.20±4.81 ^c	131.20±5.46 ^c	1188.20±5.51 ^b	294.00±5.54 ^c
Group E (garlic + half dose toltrazuril)	759±4.20 ^a	968.60±2.00 ^b	209.00±3.33 ^b	1354.40±5.21 ^a	386.20±3.81 ^a

Mean with different letters at the same column were significantly different at p<0.05

Table (2) Oocysts count/gm (OPG), lesion score, mortality % and treatment efficacy in *Eimeria* infected chickens and treated groups) n=5

Groups	One week post infection		Mortality % after one week P.I.	Two weeks Post infection		Mortality% after two weeks P.I.	Efficacy of treatment
	Oocyst/gm	Lesion score		Oocyst/gm	Lesion score		
Group B (infected non treated)	62413±1680 ^a	3.5	35%	18257 ±1291 ^a	1.9	0%	--
Group C (toltrazuril)	15749 ±692 ^c	2.5	10%	991 ±244 ^c	0.9	0%	74.9%
Group D (garlic)	41861 ±651 ^b	3.0	20%	5340 ±430 ^b	1.6	0%	32.76%
Group E (garlic +half dose toltrazuril)	15180 ±780 ^c	2.2	0%	705±131 ^c	0.9	0%	75.6%

Mean with different letters at the same column were significantly different at p<0.05

Hematological results:

In table (3), after first week P .I. the hematological studies of infected chickens in group (D) (treated with garlic) revealed no significant differences in Hb, PCV and TLC values compared with control positive group (B). Hematological studies of the group (C) (treated by toltrazuril) showed significant increase in the Hb , RBCs count and PCV values and significant decrease of TLC and MCV than the control positive group (B) at 1st week p.I. In comparable with group (E) (treated by both garlic and half dose of toltrazuril) there is a significant improvement in the Hb , RBCs count , PCV , TLC , and MCV where they reach near to the normal values compared with group A (control negative).

After two weeks P.I. those hematological parameters were significantly improved in groups C, D and E than the control positive group (B) and nearly reach to the normal values compared with group A (control negative).

(treated with toltrazuril) and (D) (treated with garlic) revealed a significant decrease in the levels of AST and ALT and a significant increase in the levels of total protein, albumin and globulin, than the group (B) (control positive). On the other hand there is no significant difference in creatinine and uric acid levels in group (C and D) compared with (B). Regarding the results of group (E) (treated with both garlic and half dose of toltrazuril), it showed a significant biochemical improvement in the values of total protein, globulin, AST, ALT, uric acid and creatinine from 1st week P.I. After two weeks P.I. these parameters were significantly improved in the liver and kidney function tests in the groups (D and C) than the control positive group (B). The group (E) showed great improvements in the 2nd week P.I which approached near the normal values compared with group A (control negative).

5. Biochemical results:

In table (4) after first week P .I. the biochemical studies of infected chickens in groups (C)

Table (3) Hematological parameters in infected chickens with Eimeria spp. (control and treated groups) (n=5)

Group	Date of sampling	RBCs x10 ⁶ /cmm	Hb/gm%	PCV%	MCV/FL	MCH	MCHC	TLC x10 ³ /cmm
Group A (Control negative)	1 st W. P.I	3.44±0.10 ^a	9.58±0.22 ^a	32.53±1.01 ^a	95.85±2.10 ^c	28.91±0.71 ^a	29.55±1.06 ^a	26.40±0.32 ^d
	2 nd W.P.I	3.82±0.26 ^a	10.38±0.61 ^a	38.43±1.29 ^a	98.47±4.74 ^c	27.17±0.68 ^b	26.15±0.96 ^b	24.2±0.13 ^d
Group B (infected non treated)	1 st W. P.I	1.95±0.06 ^d	5.49±0.29 ^d	25.17±0.99 ^b	128.99±3.31 ^a	28.35±2.03 ^a	22.13±1.96 ^b	41.34±0.12 ^a
	2 nd W.P.I	2.26±0.15 ^d	6.89±0.21 ^c	27.58±0.87 ^c	127.83±5.16 ^a	30.43±0.88 ^a	25.05±0.58 ^b	40.8±0.14 ^a
Group C (toltrazuril)	1 st W. P.I	2.57±0.11 ^c	6.72±0.17 ^c	31.68±0.81 ^a	119.14±5.36 ^{ab}	26.14±1.17 ^b	21.66±0.79 ^b	32.8±0.46 ^b
	2 nd W.P.I	2.90±0.05 ^c	9.45±0.31 ^a	32.21±0.70 ^b	111.09±2.87 ^{bc}	32.53±0.72 ^a	29.32±0.96 ^a	33.9±0.27 ^b
Group D (garlic)	1 st W. P.I	2.37±0.06 ^c	5.95±0.14 ^d	25.54±1.39 ^b	116.34±4.30 ^b	25.16±0.98 ^b	21.65±0.67 ^b	38.34±0.42 ^a
	2 nd W.P.I	2.68±0.46 ^c	8.78±0.17 ^b	32.15±0.92 ^b	119.72±3.86 ^{ab}	32.87±1.09 ^a	27.45±1.08 ^{ab}	35.8±0.46 ^b
Group E (garlic + half dose toltrazuril)	1 st W. P.I	3.06±0.09 ^b	8.01±0.22 ^b	32.95±0.48 ^a	107.85±2.53 ^b	26.29±1.27 ^b	24.33±0.68 ^b	28.30±0.12 ^c
	2 nd W.P.I	3.99±0.17 ^a	9.68±0.19 ^a	36.31±1.24 ^a	106.4±3.91 ^c	24.26±1.62 ^b	26.78±1.14 ^{ab}	30.89±0.67 ^c

Mean with different letters at the same column were significantly different at p<0.05

Table (4) Some biochemical parameters in infected chickens with *Eimeria spp.* (control and treated groups) (n=5)

Groups	Date of sampling	ALT IU/L	AST IU/L	T.prot. gm/dl	Alb. gm/dl	Globul gm/dl	U.acid mg/dl	s.creat. mg/dl
Group A (Control negative)	1 st W. P.I	19.45±0.83 ^c	46.8±0.38 ^c	4.47±0.26 ^a	3.31±0.14 ^a	1.16±0.15 ^a	8.14±0.13 ^b	0.32±0.41 ^b
	2 nd W.P.I	20.2±1.93 ^c	43.25±0.27 ^c	5.07±0.21 ^a	3.81±0.14 ^a	1.26±0.15 ^a	8.06±0.12 ^b	0.30±0.33 ^b
Group B (infected non treated)	1 st W. P.I	38.55±0.44 ^a	82.38±0.14 ^a	2.37±0.21 ^c	1.93±.17 ^c	0.44±0.56 ^c	10.92±0.35 ^a	0.45±0.26 ^a
	2 nd W.P.I	39.6±2.06 ^a	90.8±0.58 ^a	2.54±0.13 ^c	1.26±1.31 ^c	1.28±0.26 ^a	11.32±0.18 ^a	0.52±0.36 ^a
Group C (toltrazuril)	1 st W. P.I	29.45±0.26 ^b	63.8±0.58 ^b	3.12±0.16 ^b	2.16±0.16 ^b	0.96±0.53 ^b	9.94±0.14 ^a	0.42±0.24 ^a
	2 nd W.P.I	26.63±0.32 ^b	50.52±0.24 ^b	4.66±0.15 ^a	2.79±0.14 ^b	1.87±0.21 ^a	8.46±0.13 ^a	0.36±0.07 ^b
Group D (garlic)	1 st W. P.I	30.45±0.45 ^b	53.8±0.34 ^b	3.03±0.41 ^b	2.01±.25 ^b	1.0±0.16 ^b	10.42±0.52 ^a	0.40±0.56 ^a
	2 nd W.P.I	28.67±0.24 ^b	52.18±0.58 ^b	3.34±0.36 ^b	2.16±0.52 ^b	1.18±0.42 ^a	8.65±0.34 ^b	0.33±0.42 ^b
Group E (garlic + half dose toltrazuril)	1 st W. P.I	25.34±0.18 ^c	49.14±0.36 ^c	3.74±0.17 ^a	2.46±0.28 ^b	1.28±0.36 ^a	9.52±0.45 ^b	0.32±0.26 ^b
	2 nd W.P.I	21.34±0.26 ^c	45.57±0.32 ^c	4.29±0.43 ^a	2.93±0.21 ^a	1.36±0.17 ^a	8.60±0.63 ^b	0.30±0.33 ^b

Mean with different letters at the same column were significantly different at $p < 0.05$

6- Post mortem findings:

Most chickens of group B showed severe hemorrhages of the cecum, the mucosa was severely eroded, pale muscles in 1st and 2nd week P.I. In most cases of group B showed ‘cecal plugs’ consisted of the accumulation of blood, pus, oocytes and feces (Fig.1). In group (C) and (D) the chickens showed severe congestion of the mesenteric blood vessels, scattered petechial hemorrhages on subserosal layer, thick dilated hyperemic cecal wall and the fecal contents wastage with blood. In group (E) showed milder lesion than the previous groups.

7-Histopathological results:

Group B (infected): Cecum: One week P.I., the ceca showed severe congestion of mesenteric blood vessels, necrosis of the intestinal glands which replaced by different stages of *Eimeria* and caseated necrotic material. Massive hemorrhages in the lumen with desquamated epithelium and severe leucocyte infiltration mainly (heterophils, lymphocytes and eosinophils) (Fig. 2) and (Fig. 3) Most cases showed severe mucosal necrosis and severe leucocytic infiltration and destruction of the

lamina propria (Fig. 4). The cecum of chickens which were sacrificed at 2 weeks P.I., the cecum showed moderate necrosis of the intestinal glands and periglandular edema (Fig. 5). Some cecal glands were repaired with presence of some stages of *Eimeria* multifocal hyperplasia, cystic dilatation of intestinal glands were noticed.

Liver: One week P.I., the liver showed vacuolar and hydropic degeneration of the hepatocytes. Focal area of coagulative necrosis and extensive portal leucocytic aggregations mainly lymphocytes (Fig. 6). Endothelial cells hyperplasia with hyalinization of its wall and perivascular leucocytic infiltration and few fibroblasts were seen. The 2nd week P.I., the liver showed focal round cell aggregation among the hepatocytes and fibroblast proliferation around the blood vessels.

Kidneys: One week P.I., the kidneys showed severe congestion of blood vessels and round cells aggregation mainly lymphocytes and macrophages. Hydropic degeneration and coagulative necrosis of the renal tubules and interstitial hemorrhages were observed (Fig. 7).

Two week P.I, there were few focal coagulative necrosis.

Group C (toltrazuril 7.0mg/kg) Cecum: At the end of 1st week P.I., the ceca revealed moderate degenerative changes in desquamated epithelial cells mixed with few erythrocytes. Intestinal gland and epithelium contain various developmental stages of *Eimeria* spp. Extensive leucocytic infiltration in the lamina propria and submucosa with atrophy of the intestinal glands and desquamated epithelial cells the lamina propria (**Fig. 8**). Two weeks P.I., the ceca showed mild depletion of the lymphoid cells of the cecal tonsils with leucocytic infiltration and necrosis of cecal glands in lamina propria (**Fig. 9**).

Liver: One week P.I., the liver showed diffuse vacuolar and hydropic degeneration of the hepatocytes, congested blood vessels and thrombus formation in some cases. (**Fig. 10**). Focal aggregation of inflammatory cells mainly lymphocytes and heterophil were observed among the hepatocytes. The portal areas showed thickening in the bile ducts and desquamated epithelium. Two weeks P.I., liver showed thickening in the endothelial cells and fibrous tissue proliferation around the blood vessels. Multifocal coagulative necrosis and fibroblasts among the hepatocytes.

Kidneys: One week P.I., the kidneys of group C showed diffuse degenerative changes, coagulative necrosis of the renal tubules and glomerular collapse with widening of Bowman's capsule. In addition to perivascular lymphocytic infiltration and hyperplasia of the lining endothelial together with hyper-cellularity of some glomeruli and perivascular hemorrhage (**Fig. 11**). Two weeks later, the kidneys showed per vascular lymphocytic infiltration coagulative necrosis of the renal tubules and necrotic glomeruli with widening of the Bowman's capsules (**Fig. 12**).

Group D (garlic powder): Cecum: One week P.I., the ceca showed severe hemorrhages and severe destruction of intestinal villi with remnant of glands. Massive mononuclear cell infiltration and free schizonts of *Eimeria* were noticed (**Fig. 13**). Two weeks later, the cecum showed necrosis of the cecal glands with presence of few scattered stages of *Eimeria* and few erythrocytes in the mucosal layer. (**Fig. 14**). Atrophy of the cecal glands and hyaliniza-

tion of muscles were reported.

Liver: One week P.I., the liver showed vacuolar, hydropic degeneration and coagulative necrosis in addition to perivascular leucocytic infiltration mainly lymphocytes. In few cases, perivascular round cells aggregation among the hepatocytes (**Fig. 15**). Two weeks P.I. appears as normal liver.

Kidneys: The kidney of died and sacrificed chickens, one week P.I. showed multifocal cloudy swelling of the renal tubules, per vascular edema and coagulative necrosis. Some tubules were destructed and replaced by leucocytes and erythrocytes. Some glomeruli showed glomerular collapse and widening of the Bowman's capsules (**Fig. 16**). Two weeks P.I. showed less severe lesions in addition to few fibroblastic proliferation and leucocytic infiltration among the renal tubules.

Group E (garlic + toltrazuril 3.5 mg/kg): Cecum: The ceca of chickens one week P.I., showed less severity lesions than the previous groups. The epithelial layer showed mild to moderate leucocytic infiltration with intact mucosal layer and few different stages of *Eimeria* are present inside the glands with few red cells (**Fig. 17**). The sub mucosal layer showed focal necrosis of some cecal glands, mononuclear inflammatory cells infiltration and few dead oocysts present inside the cecal glands (**Fig. 18**). The lumen contained few red cells, mononuclear inflammatory cells and oocysts. Depletion of the lymphoid cecal tonsils, edema and red cells infiltration among hyalinized muscle fibers were seen. Two week P.I., the cecum of group E showed few mononuclear cells infiltration with intact epithelial layer (**Fig. 19**).

Liver: One week P.I., the liver showed mild vacuolar and hydropic degeneration and focal coagulative necrosis (**Fig. 20**). Two weeks P.I., the liver appeared abnormal liver.

Kidneys: The kidneys of chickens at the end of one week P.I., showed focal vacuolar and hydropic degeneration of the renal tubules and periglomerular edema (**Fig. 21**). Cystic dilatation of few renal tubules could be noticed. Two weeks P.I. the kidney showed as normal kidney.

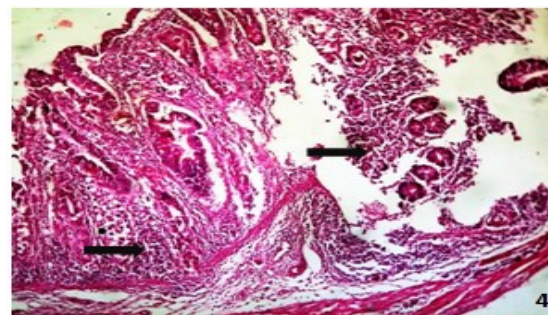
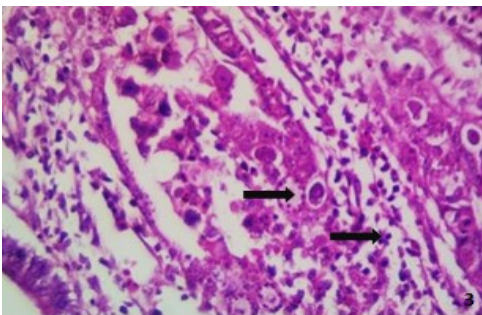
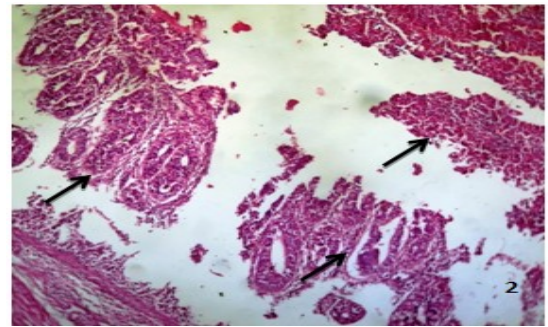


Fig. (1). Postmortem cecum of chicken in group B showing cecal plug and hyperemic thick cecal wall (lesion score+3)
 Fig. (2). Cecum of chicken in group B showing destruction of the intestinal glands, necrotic intestinal epithelial layer with presence of different stages of *Eimeria*, erythrocytes in the intestinal lumen and leucocyte cells infiltration among the glands (H&E X 200)
 Fig. (3). High power of the previous figure showing different stages of *Eimeria* and mononuclear cells infiltration in the epithelial cells. (H&E X400).
 Fig. (4). Cecum of chicken in group B showing mucosal necrosis in the intestinal epithelium with leucocytic infiltration and destruction of the lamina propria (H&E200).

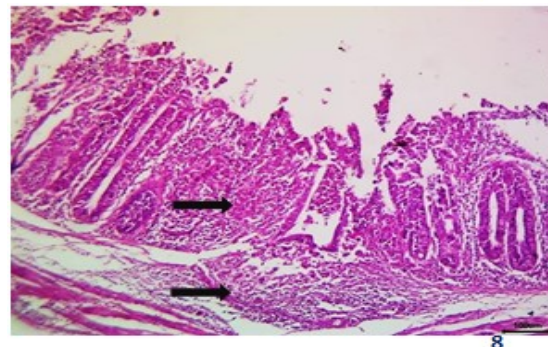
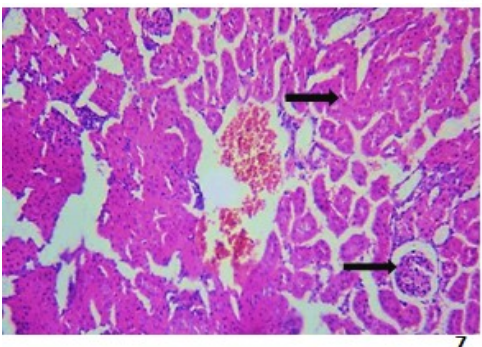
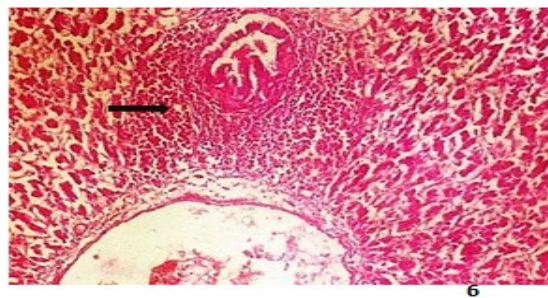
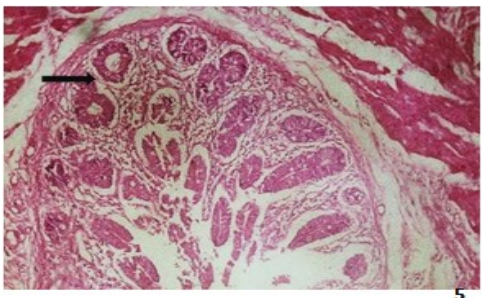


Fig. (5). Cecum of chicken in group B showing necrosis of few intestinal glands and periglandular edema (H&E X100)
 Fig. (6). Liver of chicken in group B showing focal area of coagulative necrosis, vascular congestion and extensive portal leucocytic aggregations mainly lymphocytes. (H&E X 100).
 Fig. (7). Kidney of chicken in group B showing interstitial hemorrhage and coagulative necrotic changes of renal tubules (H&E X 100).
 Fig. (8). Cecum of chicken in group C showing extensive leucocytic infiltration in the lamina propria and submucosa with atrophy of the intestinal glands and desquamated epithelial cells. (H&E X200).

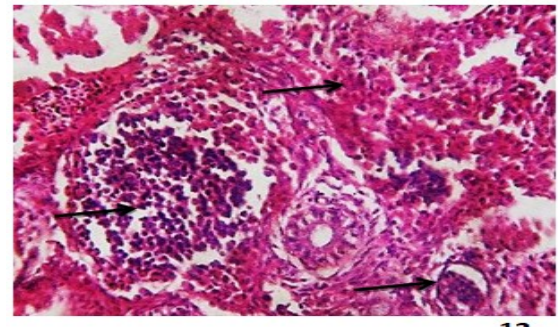
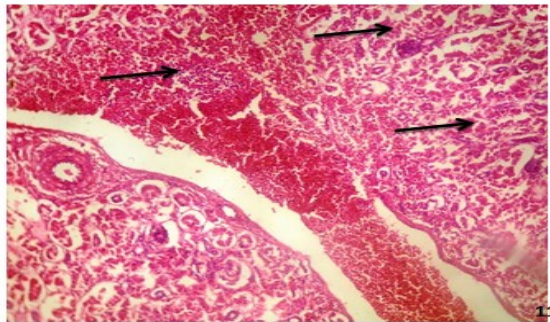
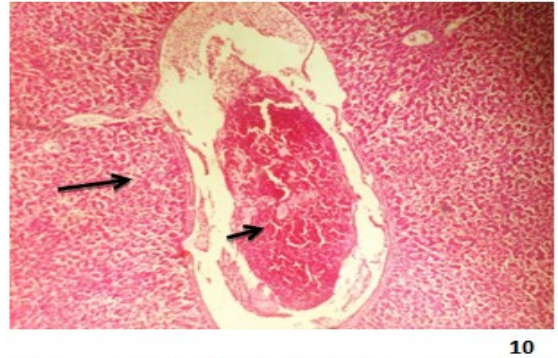
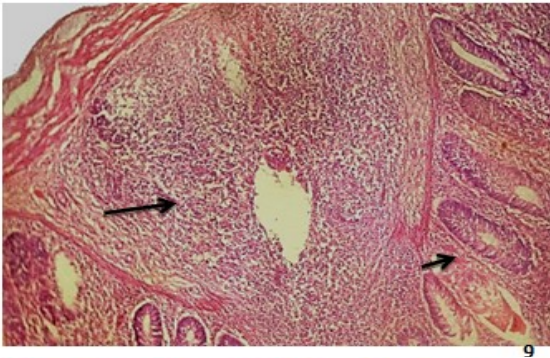


Fig. (9). Cecal tonsil of chickens in group C showing depletion of the lymphoid cells with leucocytic infiltration, edema and necrosis of cecal glands (H&E X100).

Fig. (10). Liver of chickens in group C showing diffuse vascular and hydropic degeneration, congestion and thrombus formation (H&E X100)

Fig. (11). Kidney of chicken in group C showing degenerative changes, coagulative necrosis of the renal tubules congested blood vessels and glomerular collapse with widening of Bowman's capsule. (H&E X 100).

Fig. (12). Kidney of chicken in group C showing perivascular lymphocytic infiltration, coagulative necrosis of the renal tubules and necrotic glomeruli with widening of the Bowman's capsules (H&E X200)

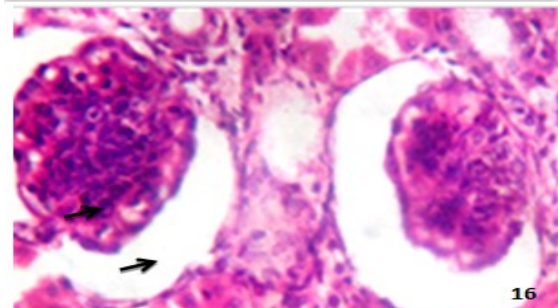
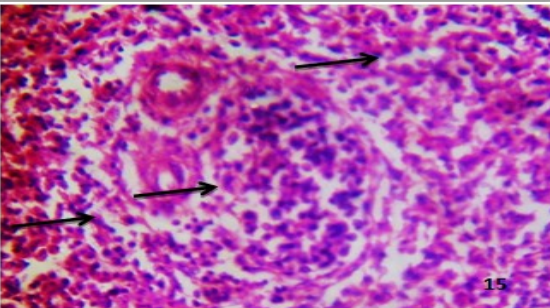
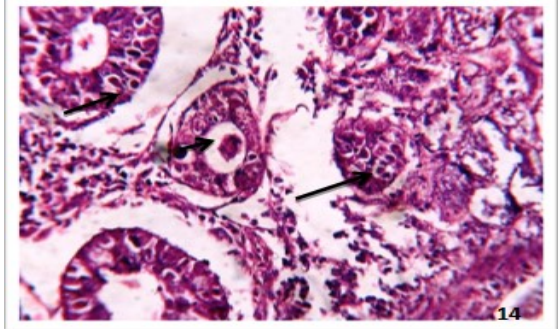
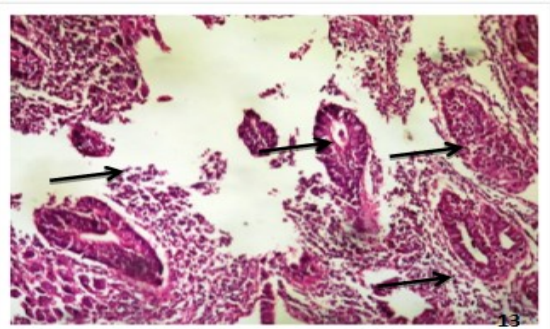


Fig. (13). Cecum of chicken in group D showing severe hemorrhage ,necrosis intestinal glands with remnant of glands ,round cell infiltration and schizonts of *Eimeria*. (H&E X200)

Fig. (14). Cecum of chicken in group D showing necrosis of the cecal glands with presence of dead of *Eimeria* oocysts in the mucosal layer. (H&E X400)

Fig. (15). Liver of chicken in group D showing degenerative changes and coagulative necrosis perivascular leucocytic infiltration mainly lymphocytes (H&E X 400).

Fig. (16). Kidney of chicken in group D showing glomerular collapse and widening of the Bowman's capsules (H& E X400)

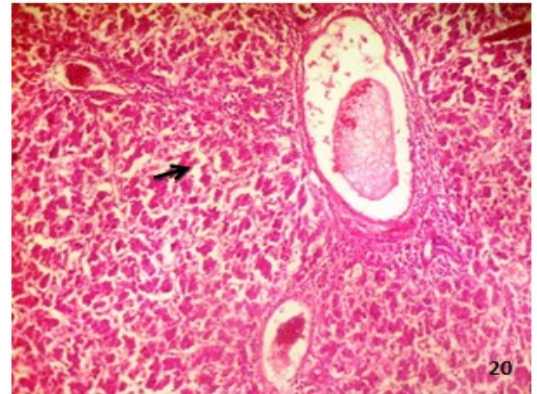
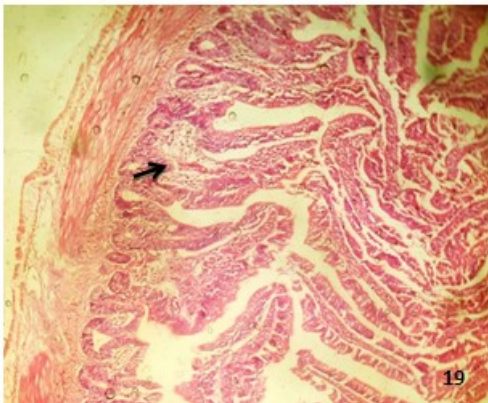
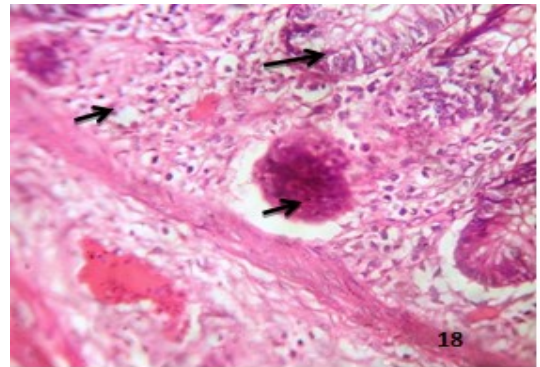
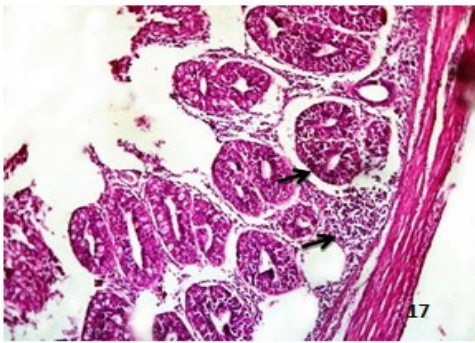


Fig. (17). Cecum of chicken in group E showing mild leucocytic infiltration and few different stages of *Eimeria* present in the glands (H&E X 100) .

Fig. (18). Cecum of chicken in group E showing necrosis of some cecal glands, round cell infiltration and few different stages of *Eimeria* present inside the cecal glands (H&EX 200).

Fig. (19). Cecum of chicken in group E showing few red and round cell infiltration (H&E X 100)

Fig. (20). Liver of chicken of group E showing mild vacuolar and hydropic degenerative changes (H&E X 100) .

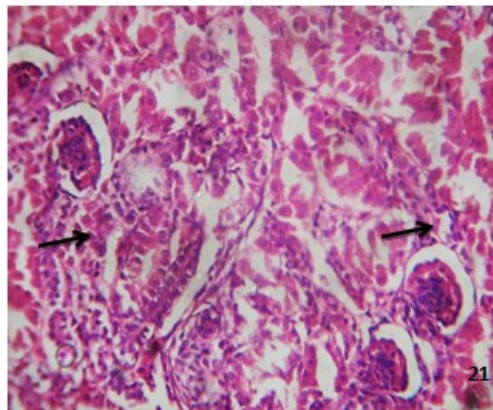


Fig. (21). Kidney of chickens at the end of one week P.I. group E showing vacuolar and hydropic degeneration of the renal tubules and peri- glomerular edema (H&E X200)

Discussion

Prevention of coccidiosis requires more alternative strategies to overcome the problem of high cost vaccines and the long duration of drug residues in the muscles and tissues (Tewari and Maharana, 2011). The current study was conducted to estimate the curative effect of toltrazuril at the recommended dose on treating *Eimeria* spp. in addition to the estimate the prophylactic effect of garlic alone or in combination with half the recommended dose of toltrazuril. Jane and Mark (2014) reported the pharmacokinetics of toltrazuril that it can cross the blood brain barrier and achieves concentration in the cerebro-spinal fluid so it is contra-indicated to pregnant mammals and also has adverse effect on the parenchymal organs. Toltrazuril is a synthetic component acts as inhibitor the parasite mitochondrial respiration and inhibition of the folic acid pathway but it has an adverse effect on the parenchyma organs (mainly liver) (Noack et al. 2019). The present study investigate the chickens infected with cecal *Eimeria* at 20 days-old which showed general clinical signs as bloody diarrhoea, ruffled feather, depression due to hemorrhagic enteritis attributed to the effect of *Eimeria* parasite which invade the intestine and retard its function. This agreed with Soulsby (1982) who said that the infected chickens with coccidiosis at three weeks old represented bloody diarrhoea by the 4th day post infection which increased at the 5th to 7th day causing 40% mortalities.

1. Body weight and weight gain:

The chickens in groups (D and E) showed significant increase of body weight during the rearing time before the experimental infection than the control group (A). These reports was attributed to growth promotion stimulant effect of garlic which was reported by El-Khtam (2014). Chickens treated against coccidiosis with garlic together with half dose of toltrazuril group (E) showed better significant effect in body weight and weight gain than those treated with full dose of toltrazuril (group C) or garlic only (group D) especially in the second week P.I., which was attributed to significant improvement in the body performance. These reports may be due to the growth promotion, im-

mune-stimulant and antioxidant effect of garlic on body performances which partially discussed by Adibmoradi et al. (2006). Allen et al. (1998) added that antioxidant components of garlic were reduced *Eimeria* replication process by ameliorating the degree of intestinal lipid per oxidation.

2-Clinical signs and mortality rate: All the treated chickens in group (E) showed gradual improvements of the symptoms of bloody diarrhea few hours after treatment and no mortalities recorded in comparing with those treated with full dose of toltrazuril (group C) (10% mortalities) or garlic only (group D)(20% mortalities) in the first week P.I. which partially discussed by Rashid et al. (2009). After the second week post infection, symptoms improved in all groups except group (B). No mortality was recorded at the second week P.I. This agreed with Soulsby (1982) and David et al. (1991).

3. Oocysts count and lesion score: The picture of OPG in groups (C) and (E) showed excellent decrease of their count. The two groups showing similar results, reduction of oocysts count and significant improvement in lesion score. Treatment efficacy of the chickening group (E) (administrated half dose of toltrazuril and garlic) revealed better results(75.6%) than both groups (C) (toltrazuril) (74.9%). The present results are in a great accordance with Mathis et al. (2004) and Rashid et al. (2009) who found that toltrazuril administered in drinking water as 7 mg /kg weight were resembling the performance of non-infected that the toltrazuril eliminate the coccidial lesion and dramatically reduce the oocysts shedding in addition to Ghanem et al. (2008) and Badrawy (2012) who reported that toltrazuril is more effective when the treatment was initiated 48 hours post infection then the medication showed full efficacy. The recommended dose of this drug decrease the oocytes output because its high potency against all the parasite stages (intracellular schizonts and gametocytes) from 1 to 168 hours post infection. Rashid et al. (2009) reported that the lower dose of toltrazuril 3.5 mg/kg and 1.75 mg/kg proved far better than the higher dose in term

of weight gain and reduction of OPG counts. The chickens in group (D) showed milder decrease of oocysts count in the first week P.I. The present study disagreed with **Adibmoradi et al. (2006)** and **Youssef et al. (2007)** who reported that the garlic had several major components that are effective against protozoa so exhibited the coccidial activity.

4. Hematological results: The chickens of group (B) (control +ve) showed a significant decrease in Hb, RBCs and PCV but a significant increase in TLC and MCV in comparable with group (A). These results go with the results that were observed by **Showkat et al. (2014)** and **Hirani et al. (2007)**. These results can be explained by presence of severe hemorrhage in cecum, bloody diarrhea and loss of appetite that leads to occurrence of macrocytic hypochromic anemia and the possibility of associated secondary bacterial infection and also increase of eosinophil number due to protozoal infestation that explain increasing TLC.

The infected chickens in group (D) (treated with garlic) after first week P.I. revealed no-significant differences in Hb, PCV and TLC values compared with control positive group (B). Where the results of Hb, RBCs, PCV, TLC and MCV were significantly improved in the groups (C) and (E) in comparable with the group (B) at the end of the experiment. These results were corresponding with that recorded by **Patra et al. (2010)** and **Showkat et al. (2014)** due to effect of therapeutic dose of the toltrazuril group (C) and dual effect of garlic and half dose of drug in group (E) which treated the hemorrhagic enteritis.

5. Biochemical results: The infected untreated chickens group (B) showed a significant decrease in total protein, albumen and globulin, while a significant increase in ALT, AST, uric acid and creatinine. These results coincide with the results that were published by **Mondal et al. (2011)** who explained that due to malabsorption, loss of appetite as result of hemorrhagic enteritis, as well as, increase of ALT, AST due to hepatocellular necrosis, and increase of uric acid and creatinine as a result of kidney dysfunction. More over; **Khalil (1995)** and **Nabil (2005)** reported heap to cellular necrosis and severe congestion of the kidneys

and hyper cellularity of the glomeruli were accompanied by coccidiosis.

In case of group (C) showed significant decrease in the levels of AST and ALT and a significant increase in the levels of total protein, albumin and globulin at 1st and 2nd week P.I. But the levels of kidney function were non-significantly changed in comparing with control positive group (B). These results may be attributed to adverse reaction of the drug that results have emphasized partially with the results of **Noack et al. (2019)** and **Wenlong et al. (2019)** who report that toltrazuril induce focal hepatocellular necrosis, slight renal tubule protein casts and alveolar wall thickening.

With regard to the group (D) there was a significant decrease of ALT, AST, total protein, and albumen and globulin levels mainly in the 1st week P.I. Creatinine and uric acid were non-significantly changed in comparing with control positive group (B) (**Gedic et al. 2005**).

While these parameters in groups (E) (treated with both garlic and half dose of toltrazuril) showed a significant biochemical improvement in the values of total protein, albumen, globulin, AST, ALT, uric acid and creatinine from 1st week P.I till the end of the observation, which approached near the normal values. These results were in agreement with **Hassan et al. (2009)** and **Jafari et al. (2012)** who explained these improvement may be attributed to the anticoccidial effect of both the drug and garlic in addition to the anti-inflammatory, antioxidant and immune modulator effect of the garlic. We had partial credibility with **Rashid et al. (2009)** who reported that the lower dose of toltrazuril 3.5 mg/kg was good effectiveness against coccidiosis.

6. Pathological results: The chickens of group (B) (control positive) showed severe hemorrhagic enteritis with replacement of the cecal glands with the stages of *Eimaria* and mucosal necrosis in the intestinal epithelium with leucocyte infiltration and destruction of the lamina propria. Coagulative necrosis focal leucocytes aggregations in liver and kidneys were reported. These results have great accordance with **Khalil (1995)** **Nabil (2005)** and **Badrawy (2012)**. In our opinion the degenerative changes of liver and kidneys were ex-

plained by **Khalil (1995) and Salah (1998)** due to dysfunction of intestine and severe blood loss causing significant decrease in serum total protein level and due to the high level of toxins released by the protozoa in blood with lack of the detoxifying function of liver **Lakkundi et al. (2002)**.

In groups (C) and (E) the chickens showed mild to moderate enteritis, scattered hemorrhages, leucocytic infiltration and few different stages of *Eimeria* present in the glands. These results were explained by **Badrawy (2012) and Ghanem et al. (2008)** who attributed these to the toltrazuril treated effect. The chickens of group (D) which take the garlic powder as prophylaxis showed moderate improvement in the intestinal lesions than untreated group (B) which supported by **Gedic et al. (2005)**. In the present study, the liver lesions of group (C) which administered the high dose of toltrazuril showed interstitial lymphocytic infiltration, severe congestion and severe degenerative changes and showed severe congestion and focal mononuclear cell infiltration together with diffuse severe necrosis and hyper cellularity of glomeruli in the kidneys. That lesions had an agreement with **Wenlong et al. (2019)** who report that toltrazuril induce focal hepatocellular necrosis, slight renal tubule protein casts and alveolar wall thickening.

Group (E) (treated with garlic and half dose of toltrazuril) showed mild to moderate inflammatory infiltrates, with intact mucosal layer and few different stages of *Eimeria* are present inside the glands with few red cells in the 1st week then the cecum became apparently normal in the 2nd week P.I. Also the liver and kidneys showed milder lesions. These results proved the suggestion that the garlic powder exhibit the harmful effect of toltrazuril on the internal organs. **El-khtam et al. (2014)** explained the anticoccidial activity of garlic due to the antioxidant rich plant that may be lethal to the parasite by including oxidative stress and neutralize reactive oxygen.

It could be concluded that:

The results in the present study suggest that supplementation of garlic powder in concentration 8.0 mg/kg ration has moderate beneficial effect of prophylaxis against cecal *Eimeria*.

Toltrazuril is a good coccidiostate (74%

treatment efficacy) reduce the mortalities and oocysts count, but the combination between garlic powder as prophylaxis in ration with half dose of toltrazuril 3.5 mg/kg weight exhibits significant anticoccidial activity (75% treatment efficacy), prevent the mortalities, improve the body condition. So, they recommended for controlling chicken coccidiosis to reduce its adverse effect on liver and kidneys.

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