

THE EFFECT OF DICLAZURIL AND MADURAMICIN ALONE OR TOGETHER IN CONTROLLING CECAL COCCIDIOSIS IN CHICKENS

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ABSTRACT

Coccidiosis in chicken farms are considered as one of the most serious problems affecting both small and large poultry producers. Eimeria tenella now is consider as one of the most pathogenic and important Eimeria species affecting about 90% of intensive broiler production. This study was carried out to evaluate the efficacy of maduramicin (5ppm), diclazuril (2.5ppm) and both drugs together on healthy and experimentally infected chicken with E.tenella (50000 sporulated oocyst) on 15th day of age after the symptoms of infection had appeared on 5th day of infection for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day of age. The results revealed that the infected group treated with both of maduramicin and diclazuril did not show any antagonistic interaction between them but leading to the best results among all infected treated groups by overcome clinical signs, decrease mortality rate, lesion scoring, give the lowest count of oocyst shedding and represented the best improvement in biochemical parameters throughout the experiment. This reflects the synergistic interaction between them without any significant adverse effect. The efficacy of diclazuril was superior to maduramicin in treatment of cecal coccidiosis by marked improvement on cecal integrity and reduction of the oocyst shedding. Non infected treated groups did not show any significant difference than negative control group during the experimental period and this reflects the safe use of both drugs either individually or together.

INTRODUCTION

As the world poultry production continues to grow, so do concerns about the control of coccidiosis, which remains one of the most commonly reported diseases of chickens (*Biggs, 1982 and Xie et al., 2001*). Coccidiosis is caused by obligate intracellular protozoan parasites of genus *Eimeria* lives and multiplies in the intestinal tract and causes tissue damage which interfere with food digestion and nutrient absorption as well as causing dehydration, blood loss and expose the bird to bacterial infections, like clostridium and salmonella. Cecal coccidiosis caused by *Eimeria tenella*, manifested clinically by bloody mucoid drooping and constitutes a significant economic impact in poultry. Lower weight gain, reduced feed efficiency, poor performance of surviving birds, mortality, routine prophylactic and therapeutic medication are important cost factors (*Danforth and Augustine., 1985 and Yun et al., 2000*).

For controlling of this disease, effective therapeutics must be given specially those originated from new chemical components and showed successful anticoccidial effect for infected host. Diclazuril is a synthetic molecule belongs to benzeneacetonitrile derivatives. The efficacy of diclazuril in prevention and control of coccidiosis in broiler chickens was extensively studied without development of drug resistance (*Amer et al., 2007*). Maduramicin ammonium is monoglycoside polyether ionophore produced by fermentation from *Actinomadura rubra* fungus and exhibited a high degree of anticoccidial activity (*Berger et al., 1988*).

The objective of this work was to study the effect of maduramicin and diclazuril interaction on healthy and experimentally infected chickens with *E.tenella* in order to achieve the optimal control to cecal coccidiosis. Evaluate their effectiveness as anticoccidials in improving clinical signs, oocyst counting, lesion scoring and mortality rate and their effect on the biochemical parameters, were studied.

MATERIALS AND METHODS

Drugs:

Diclazuril (Diclosol®) new formulation of water soluble diclazuril developed and described by Pharma Swede-Egypt. Each 1 ml of diclosol liquid contains 10 mg of diclazuril used at dose of 1ml / 4 liters (2.5ppm).

Maduramicin ammonium (Coxacin 1 %®) broad spectrum ionophore anticoccidial premix for broiler developed and produced by Unipharma. Each 100 gm of coxacin 1% premix contain 1gm of maduramicin ammonium used at dose of 500gm / ton (5ppm).

Experimental chickens:

One hundred and sixty, one-day old unsexed avian 48-broiler chicks were obtained from STAR FARM (Abd El Salam Hegazy), Tanta, Egypt. They divided into 8 equal groups each contains 20 chicks and housed in a well-isolated floor pens, kept separately using wooden partitions and reared under complete hygienic conditions. Water and food were provided *ad libitum* during the experimental period (42 days) using standard broiler ration free from any anticoccidial drugs obtained from FATY HENS company. Vaccination was carried out against both New castle and Gumboro diseases.

Grouping and experimental design:

- Group 1:** Non infected, non treated and lets as negative control.
- Group2:** Non infected, given recommended dose of maduramicin (5 ppm) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day in the ration.
- Group3:** Non infected, treated with therapeutic dose of diclazuril (2.5 ppm) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day in drinking water.
- Group4:** Non infected, given both recommended dose of diclazuril (2.5ppm) and maduramicin (5 ppm) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day.
- Group 5:** Infected with *E.tenella* (50.000 oocysts / bird) on 15 day of age, non treated and considered as positive control.
- Group 6:** Infected with *E.tenella* (50.000 oocysts / bird) on 15 day of age and given maduramicin (5ppm) after the symptoms of infection had appeared (on 5th day of infection) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day in the ration.
- Group 7:** Infected with *E.tenella* (50.000 oocysts / bird) on 15 day of age and treated with diclazuril (2.5 ppm) after the symptoms of infection had appeared (on 5th day of infection) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day in drinking water.
- Group 8:** Infected with *E.tenella* (50.000 oocysts / bird) on 15 day of age, given both diclazuril (2.5ppm) and maduramicin (5 ppm) after the symptoms of infection had appeared (on 5th day of infection) for 3 successive days from 20th day to 22nd day and from 30th day to 32nd day.

Preparation of *E.tenella* inoculum and experimental infection:

Oocysts of *E.tenella* were obtained from caeci of naturally infected chickens then separated by using sieving and sedimentation techniques. These oocysts were put in 2.5% potassium dichromate for sporulation in suitable temperature. The resulted sporulated oocysts were cleared and counted each ml of the solution by using Mc-Master technique *Soulsby (1978)*. Each bird of 4 infected groups was inoculated directly into the crop with 1ml of solution containing 50,000 of sporulated oocysts on the 15th day of age using rubber syringe after opening of chick's mouth and holding its neck backward according to *Saber (1995)*.

Evaluation of the anticoccidial efficacy:

- a) **Clinical signs:** Include mainly bloody dropping, diarrhea, stop feeding, congested cloacae and depression *Brander et al., (1991)*. Chickens after infection and treatment were kept under observation for recording the clinical signs.
- b) **Lesion scoring:** Five birds were killed one-week post infection and gross lesions of the infected chickens' ceci were taken as criterion to detect the severity of infection in medicated groups to be compared with those of non medicated ones and a scoring system was adopted between 0 and 4 according to *Johnson and Reid, (1970)*. The ceci were examined to insure that the produced lesions are from coccidiosis by microscopic examination of mucosal scrapings from the site of lesions to show the presence of oocysts or schizonts.

c) Oocyst count: Representative freshly voided dropping samples were collected from infected groups daily through 5 floor areas in X manner/meter squares for 11 days from the 6th day after infection (21st day of age) to (31st days of age). Number of oocysts/gm of feces counted by using the Mc Master counting chamber according to *Abd El-Rahman et al., (1982)*.

d) Mortality rate: Mortality was recorded during out the experimental period and the exact cause of mortality was confirmed by postmortem examination.

Biochemical analysis:

On 21st, 28th, 35th and 42nd day of age blood samples were collected (5 birds / group), samples of 5ml were collected in clean dry centrifuge tube without anticoagulant and left in sloap position to clot at room temperature, then centrifuged for about 10 minutes at 3000 rpm to obtain clear supernatant serum.

1- Liver function tests: Colorimetric determination of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) according to *Reitman and Frankel, (1957)*, Alkaline phosphatase activity (ALP) according to *Kind and King, (1954)*, total serum protein according to *Henry, (1964)*, serum albumin according to *Doumas et al., (1971)*, serum globulin by subtracting serum albumin value from total serum protein value.

2- Kidney function tests: Colorimetric determination of serum uric acid according to *Barham and Trinder, (1972)* and serum creatinine according to the method of *Henry, (1974)* were used.

Statistical analysis:

Analysis of variance (ANOVA) was used to compare the means of values of the various groups at a significance level of $P < 0.05$. Statistical analysis was performed using the method of *Petrie and Watson, (1999)* and computerized using *SPSS 11 (2001)*.

RESULTS

Anticoccidial efficacy:

a- Clinical signs:

The daily observation of the chickens of infected non treated group showed sick bird performance (ruffled feathers, huddling, depression), loss of appetite and intensive bloody diarrhea began on the 5th day post infection. In infected group given maduramicin chickens were apparently normal but in the first week, there was a mild depression and slight discoloration of feaces. Infected treated groups with diclazuril or both drugs signs subsided by introducing the medication and chickens were more apparently healthy during this period. There were no noticeable differences between the treated groups without infection and negative control group allover the experiment.

b- Lesion scoring:

The caeci of the infected non treated (positive control) group showed the highest lesion scores ranged from 3+ to 4+ one week post infection (P.I). The lesion scores were improved by maduramicin showed +2 lesion score, diclazuril and both drugs together showed +1 lesion score.

c- Oocyst counting:

The infected non treated control group showed the highest oocyst count shedding reach its maximum count on the 7th day post infection. There were apparent differences in decreasing the oocyst count between infected treated groups specially infected group treated with both diclazuril and maduramicin showed the lowest oocyst count (Table 1).

Table (1): Oocyst shedding in infected group (5), infected controlled by maduramicin group (6), infected treated by diclazuril group (7), infected treated by both drugs group (8).

	Infected groups oocyst count (x 10 ³) /gm faeces				ANOVA	
	G 5	G 6	G 7	G 8	f	P-value
21 day	333.40±29.46 ^a	146.00±9.62 ^b	109.40±15.87 ^b	103.80±4.49 ^b	190.920	<0.001*
22 day	526.20±21.38 ^a	244.60±33.00 ^b	183.00±18.57 ^b	146.00±9.62 ^b	296.718	<0.001*
23 day	336.60±14.93 ^a	186.00±39.27 ^b	157.00±5.70 ^b	115.60±17.16 ^b	88.658	<0.001*
24 day	248.20±15.21 ^a	120.20±13.61 ^b	106.00±11.94 ^b	67.60±16.01 ^b	150.605	<0.001*
25 day	124.00±27.01 ^a	62.60±9.02 ^b	52.80±5.89 ^b	37.00±5.70 ^b	33.048	<0.001*
26 day	57.00±4.58 ^a	29.40±6.77 ^b	25.80±3.96 ^b	17.60±1.95 ^b	67.729	<0.001*
27 day	18.60±4.67 ^a	10.20±1.79 ^b	9.60±1.14 ^b	4.40±0.62 ^b	25.877	<0.001*
28 day	5.46±1.20 ^a	3.01±0.19 ^b	2.76±0.73 ^b	2.51±1.01 ^b	12.347	<0.001*
29 day	3.17±0.73 ^a	2.19±0.48 ^b	0.00±0.00 ^b	0.00±0.00 ^b	3.081	0.047
30 day	1.31±0.48 ^a	0.66±0.11 ^b	0.00±0.00 ^b	0.00±0.00 ^b	5.609	0.008*
31 day	1.00±0.16 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	200.000	<0.001*

Data represented as mean ± SD with different superscripts showed significant difference at P<0.05 using ANOVA test.

d- Mortality rate:

The infected non treated group showed a high significant mortality rate (25 %). The mortality rate of all treated groups was significantly decreased to 5%, 10% and 10 % for groups treated by maduramicin, diclazuril and both drugs together respectively. All cases of mortality

occur in group 5 on (6th, 8th, 10th, 11th and 13th) days post infection (P.I), group 6 on (11th) day (P.I), group 7 on (6th and 8th) days (P.I) and in group 8 on (4th and 8th) days post infection.

Gross morphological picture: Showed that the ceaci of infected non treated group in early stage were dilated (ballooned), their mucous membrane assumed a red colour easily visible even on the surface of the serosa filled with blood and tissue debris. Then the caecal walls were thickened, hemorrhagic and there is a mass of clotted blood in the caecal lumen collected together with ulcerated mucosa to form a core with the presence of erosions in the caecal mucosa. In latter stage (8th days post infection) showed accumulation of varying quantities of caseous necrotic material in the ceaci with pin point grayish nodules as aggregation of oocysts increased in volume and become rigid. Ceaci were whitish in color and of rod like consistency.

Biochemical findings:

The infected non treated group elicited a significant increase in serum activity of AST (Table 2), ALT (Table 3), ALP (Table 4), serum uric acid (Table 8) and creatinine (Table 9) and a significant decrease in total serum protein (Table 5), serum albumin (Table 6) and globulins (Table 7). Infected treated groups elicited a significant decrease in serum activity of AST, ALT, ALP, serum uric acid and creatinine and a significant increase in total serum protein, serum albumin and globulins. Group 6 given maduramicin showed less improvement than other two treated groups. Group 8 treated with both drugs together appeared nearly as negative control group. No significant differences were recorded between non infected treated groups when compared with negative group.

Table (2): Showed AST in all groups.

Group Age	Aspartate aminotransferase (AST U/L)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	42.31 ±1.44	45.76 ±3.40b	47.16 ±3.05b	45.62 ±3.14b	89.23 ±8.84c	79.95 ±0.96ab	69.20 ±1.30abd	66.67 ±0.95abef	196.123	<0.001*
28day	46.60 ±2.73	44.00 ±2.51b	43.56 ±3.70b	43.94 ±1.73b	81.60 ±7.89c	70.25 ±2.73ab	55.13 ±4.06ad	50.80 ±4.50bef	84.710	<0.001*
35day	44.64 ±3.65	44.99 ±3.28b	46.72 ±1.67b	43.75 ±3.11b	72.66 ±2.95c	64.92 ±2.83ab	53.00 ±1.66abd	48.68 ±1.32bef	128.208	<0.001*
42day	44.07 ±3.32	40.63 ±0.46b	41.26 ±0.72b	43.67 ±0.14b	67.81 ±3.49c	53.55 ±2.67ab	51.05 ±5.28ab	45.85 ±1.29bef	98.401	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (3): Showed ALT in all groups.

Group Age	Alanine aminotransferase (ALT U/L)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	45.67 ±3.36	42.93 ±2.03b	45.07 ±2.95b	44.52 ±2.16b	95.54 ±1.48c	88.74 ±1.05ab	80.80 ±3.00abd	68.57 ±0.73abef	537.947	<0.001*
28day	44.77 ±2.94	44.44 ±0.26	44.61 ±3.13	45.11 ±3.15	88.85 ±9.70c	69.67 ±5.34ab	54.27 ±21.91abd	52.00 ±3.50abe	1.214	<0.001*
35day	41.10 ±1.01	42.38 ±1.85b	40.95 ±0.90b	46.76 ±1.99ab	78.58 ±0.15c	65.60 ±4.08ab	53.73 ±2.20abd	49.76 ±0.63abef	343.560	<0.001*
42day	44.75 ±2.94	42.31 ±0.40b	42.08 ±0.16b	40.89 ±0.85b	67.84 ±2.30c	54.01 ±3.81ab	51.23 ±3.20ab	48.01 ±2.39bef	70.929	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (4): Showed ALP in all groups.

Group Age	Alkaline phosphatase (ALP U/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	43.92 ±0.94	44.17 ±0.96b	43.23 ±1.68b	45.90 ±1.50b	93.27 ±0.99c	86.41 ±3.59ab	79.70 ±0.89abd	76.84 ±0.89abe	850.692	<0.001*
28day	44.08 ±1.35	43.93 ±1.82b	42.05 ±2.07b	45.62 ±2.35b	90.66 ±1.24c	80.60 ±1.24ab	76.51 ±8.91ab	67.53 ±1.41abef	156.210	<0.001*
35day	45.42 ±2.13	44.45 ±1.69b	42.50 ±2.27b	44.08 ±1.17b	84.12 ±2.06c	72.92 ±1.79ab	57.30 ±2.58abd	58.60 ±2.70abe	298.880	<0.001*
42day	44.51 ±0.60	42.01 ±0.89b	43.02 ±0.86b	43.01 ±1.17b	75.60 ±1.47c	62.86 ±2.19ab	48.92 ±2.35abd	43.88 ±2.66abef	404.930	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (5): Showed total serum protein in all groups.

Group Age	Alkaline phosphatase (ALP U/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	3.58 ±0.22	3.55 ±0.08b	3.59 ±0.07b	3.57 ±0.18b	2.30 ±0.20c	2.44 ±0.15a	2.61 ±0.27a	2.77 ±0.14abe	53.049	<0.001*
28day	3.63 ±0.17	3.62 ±0.14b	3.65 ±0.12b	3.59 ±0.14b	2.39 ±0.34c	2.69 ±0.15a	2.80 ±0.20a	2.87 ±0.36abe	26.878	<0.001*
35day	3.67 ±0.10	3.66 ±0.19b	3.70 ±0.16b	3.71 ±0.19b	2.48 ±0.23c	2.74 ±0.14a	3.02 ±0.45ad	2.96 ±0.07ab	25.512	<0.001*
42day	3.69 ±0.14	3.75 ±0.13b	3.72 ±0.13b	3.67 ±0.17b	2.74 ±0.19c	3.05 ±0.25a	3.39 ±0.14bd	3.20 ±0.37ab	16.557	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (6): Showed serum albumin in all groups.

Group Age	Serum albumin (g/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	1.94 ±0.14	1.92 ±0.06b	1.86 ±0.10b	1.95 ±0.10b	1.17 ±0.14c	1.31 ±0.07a	1.33 ±0.10a	1.44 ±0.33a	23.900	<0.001*
28day	2.00 ±0.06	2.01 ±0.05b	2.04 ±0.14b	1.95 ±0.12b	1.25 ±0.11c	1.41 ±0.12a	1.41 ±0.24a	1.55 ±0.37a	16.518	<0.001*
35day	2.02 ±0.08	1.97 ±0.03b	2.05 ±0.06b	1.98 ±0.12b	1.32 ±0.19c	1.54 ±0.26a	1.61 ±0.37a	1.60 ±0.13a	12.965	<0.001*
42day	1.94 ±0.10	1.93 ±0.14	2.04 ±0.04	1.99 ±0.02	1.52 ±0.09	1.71 ±0.31	1.86 ±0.30	1.74 ±0.66	1.774	0.127

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (7): Showed serum globulins in all groups.

Group Age	Serum globulins (g/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	1.64 ±0.13	1.63 ±0.05b	1.73 ±0.10b	1.62 ±0.22b	1.13 ±0.13c	1.13 ±0.21a	1.28 ±0.19a	1.33 ±0.25	9.186	<0.001*
28day	1.63 ±0.11	1.61 ±0.09b	1.61 ±0.09b	1.64 ±0.14b	1.14 ±0.25c	1.28 ±0.14a	1.39 ±0.09	1.32 ±0.26	7.381	<0.001*
35day	1.65 ±0.14	1.69 ±0.20b	1.65 ±0.11b	1.73 ±0.13b	1.16 ±0.10c	1.20 ±0.12a	1.41 ±0.11d	1.36 ±0.09	14.706	<0.001*
42day	1.75 ±0.15	1.82 ±0.07b	1.68 ±0.10b	1.68 ±0.18b	1.22 ±0.16c	1.34 ±0.15a	1.53 ±0.23d	1.46 ±0.31	6.744	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (8): Showed serum uric acid in all groups.

Group Age	Serum uric acid (mg/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	6.74 ±0.54	6.60 ±0.32b	6.93 ±0.09b	6.64 ±0.38b	9.91 ±1.76c	8.69 ±0.42a	7.58 ±0.34bd	7.31 ±0.59be	8.971	<0.001*
28day	6.29 ±0.19	6.59 ±0.52b	6.21 ±0.61b	6.31 ±0.13b	9.23 ±0.45c	7.89 ±0.65ab	7.29 ±0.39ab	7.34 ±0.40ab	26.361	<0.001*
35day	5.79 ±0.35	6.90 ±0.36ab	6.00 ±0.26b	6.65 ±0.21ab	8.33 ±0.29c	7.54 ±0.34ab	6.87 ±0.59abd	6.60 ±0.40abe	24.725	<0.001*
42day	5.31 ±0.24	5.69 ±0.11b	5.69 ±0.21b	5.89 ±0.12ab	7.58 ±0.56c	6.83 ±0.21ab	5.93 ±0.24abd	5.29 ±0.21bef	42.339	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

Table (9): Showed serum creatinine in all groups.

Group Age	Serum creatinine (mg/dl)								ANOVA	
	G 1 Non Infected	G 2 Non Infected + M	G 3 Non Infected + D	G 4 Non Infected +M+ D	G 5 Infected	G 6 Infected + M	G 7 Infected + D	G 8 Infected +M+ D	f	P-value
21day	0.80 ±0.12	0.80 ±0.07b	0.82 ±0.03b	0.79 ±0.07b	1.64 ±0.18c	1.07 ±0.14ab	0.99 ±0.10b	0.92 ±0.02b	36.777	<0.001*
28day	0.70 ±0.07	0.83 ±0.08b	0.79 ±0.06b	0.76 ±0.04b	1.46 ±0.19c	0.94 ±0.11ab	0.81 ±0.02b	0.66 ±0.03bef	39.688	<0.001*
35day	0.69 ±0.07	0.81 ±0.11b	0.79 ±0.07b	0.78 ±0.07b	1.14 ±0.08c	0.82 ±0.09b	0.72 ±0.16bd	0.64 ±0.12efb	11.191	<0.001*
42day	0.75 ±0.05	0.77 ±0.03	0.76 ±0.07	0.77 ±0.06	0.92 ±0.12	0.74 ±0.17	0.64 ±0.15bd	0.57 ±0.07bef	5.203	<0.001*

D= Diclazuril

M= Maduramicin

Data represented as mean ± SD.

Significant at P<0.05 using ANOVA test.

DISCUSSION

Infected non treated chickens showed severe clinical manifestations such as anorexia, depression, loss of appetite, hypopigmentation, severe continuous bloody dropping and hemorrhagic feces around the cloacae, such findings were in agreement with *Hofstad et al., (1984), Allam, (1989) and Saif et al., (2003)* about common signs of ceccal coccidiosis. The bloody diarrhea was recorded in infected groups with *E.tenella* on the 5th day post infection (P.I), these signs subsided gradually with observation of few discolored droppings and varying degrees of depression until the end of the experiment, this agreed with what is observed by *Conway and Mckenzie (1991)*. Appearance of typical clinical signs due to the used infective dose coordinated with *Holdsworth et al., (2004)* about the conventional methods for evaluating drug efficacy involve challenging birds with isolates of *Eimeria* obtained from the field using a sufficiently large dose of oocysts to induce lesions in susceptible birds, adversely affected the general state of health, dropping change and average mortality. Such studies can indicate if the drug is able to control an infection capable of causing clinical disease. Infected chickens controlled with maduramicin at (5ppm) in feed showed mild depression and slight dropping discoloration which improved at the end of the 1st week, the used dose agreed with *McDougald et al., (1987)* who reported that control of coccidiosis by maduramicin was significant at 4 ppm but best at 5-7 ppm. Infected chickens treated with diclazuril at (2.5 ppm) given after the symptoms of infection had appeared (on 5th day of infection) the drug treatment lasted for three successive days as

recommended by *Verheyen et al., (1988)* who declared that a marked inhibition of merozoites formation was observed and a complete necrosis of schizonts occurred (after 3 days of treatment) inhibiting later phases of differentiation. Drug used in drinking water, as recommended by *Reid, (1990)* who reported that if signs of the disease appear and birds getting sick, they lose their appetite. Therefore, soluble medication should be provided in the drinking water and use drugs that are appropriate for coccidia's late life cycle. Treated chickens were apparently normal with a good activity, this result agreed with *Kwazone and Fabio (1994); El-Banna et al., (2005) and El-Dakhly et al., (2006)* who confirmed that diclazuril solution induced a marked activity in stopping the cycle of coccidial development inside the medicated birds especially when applied on the day of the first blood appearance in bird's drooping and that is recorded by *Jiang et al., (1997)* about no blood dropping and few oocysts were discharged in diclazuril treated chickens. Infected chickens controlled by both drugs were apparently healthy with normal appearance and activity throughout the experiment, this could be attributed to the good effect of both drugs in overcome the clinical signs. Non infected treated groups with maduramicin, diclazuril or both drugs together did not show any characteristic changes in normal activity or appearance of treated chickens than non treated birds without infection.

Disease severity is judged by criteria including cecal lesions, fecal oocyst count and mortality rate. In the present study it has been shown that the oocyst output of the infected non treated group was greatly increased with heavy lesion scores from +3 to +4 and high mortality rate.

Different grades of lesion scores could be reflect the individual response to infection and/or treatment and explained by various levels of birds' immunity to cecal coccidiosis infection and this opinion supported by **Long et al., (1980) and Clare et al., (1985)**. Variable mortality resulting from invasion of massive number of infective and replicative stages of the parasite as obtained by **Yun et al., (2000)** and confirmed by the high number of oocyst count recorded in this group, these findings coordinated with the findings of **El-Dakhly et al., (2006)**. Infected group controlled with maduramicin showed a significant reduction in oocyst output when compared with infected non treated group and improved lesion scores of infected cecum, these findings agree with **Conway et al., (1995); Zarate et al., (1998) and Arafa et al., (2004)** investigated that maduramicin was effective in reducing lesions and mortality. Mortality rate decreased from 25% of infected non treated group to 5 % of infected group treated with maduramicin, the occurrences of mortalities mentioned by **Shakshouk, (1996)** who recorded 1.67 % mortalities among the group challenged and medicated with maduramicin and **McDougald et al., (1990)** who reported that mortality from coccidiosis averaged 11.9% in unmedicated controls, compared with 0.6% with 4 ppm of maduramicin or no mortality with 5-7 ppm. The discrepancy between the present investigation and other authors may be due to drug resistance or other immunological factors. This improvement in treated birds was obtained by **Cerruti Sola et al., (1996)** who recorded better performances and less severe intestinal damages as the replication and the development of the parasite in the intestinal mucosa were impaired and delayed by using maduramicin (5 ppm).

Infected chickens treated with diclazuril water soluble formulation at 1 ml/ 4 liters of the drinking water showed a significant decrease in the number of oocyst shedding and greater suppression of lesion scores when compared with infected non treated group, it also had the ability to reduce the mortality rate. These results agree with *El-Dakhly et al., (2006) and Amer et al., (2007)* who reported that diclazuril in drinking water at (2.5 ppm) was appropriate for use in prevention and treatment of *Eimeria tenella* infection in chickens as indicated by decreasing number of total oocyst count through reduction in fecal shedding, decreasing lesion score and increasing survival rate in treated groups and also based on the findings of *Maes et al., (1988)* who found that diclazuril not only interrupts the life cycle of *E.tenella* at a very early stage but also acting on the sexual stages as well. Infected group treated with both drugs in both recommended dose showed a great improvement in lesion score and more significant decrease in total oocyst count than other two treated groups throughout the experiment till the end of the oocyst shedding and these results similar to *Zarate et al., (1998)* about diclazuril and maduramicin in producing good improvement in lesion scoring. Suppression of oocyst production is one of the most useful parameters for evaluation the efficacy of anticoccidial drugs as mentioned by *Long et al., (1975), Braunius, (1980) and Voeten and Braunius, (1981)* that oocyst counts give an accurate measure of infection induced in the birds at the time of sampling. Non infected treated groups with maduramicin, diclazuril or both drugs together showed normal internal organ during the post mortem examination and all non infected groups did not record any mortality.

Serum biochemical analysis of the infected non treated group revealed impairment of liver functions expressed as a significant decrease in total serum protein, serum albumin, globulins and a significant elevation in serum activity of AST, ALT and Alkaline phosphatase. These results may be probably due to liver damage or other factors such as loss of appetite, bloody diarrhea, sloughing of mucosal cells in the cecum which caused liberating of the intracellular enzymes **Rouiller, (1964)**. Results are in agreement with **Musajev and Surkova (1970)** who reported that infection with *E.tenella* decrease the protein metabolism in liver. Also degenerative changes in liver lead to hypoalbuminemia (the largest protein fraction of avian blood) and decreased globulins (except gamma globulin) that finally lead to decreased total proteins **Coles, (1986) and Schalm, (1986)**. Also *E.tenella* infection disturbed the kidney functions which expressed as a significant elevation in serum uric acid and creatinine when compared with the non infected control group, these results coordinated with **Halliwell (1981) and Chandra et al., (1983)**. These disturbances in kidney functions similar to what is mentioned by **Saad (1998)** who explained that this significant increase in serum uric acid and creatinine indicating renal damage and could be attributed to the circulating toxins produced from infection with coccidia. The previous results confirmed also by **Singh et al., (1976), Dahshan, (1996) and Ibrahim, (1998)** who reported that infected chickens with *E.tenella* developed hepatocellular and renal damage accompanied with a significant elevation in serum AST, ALT,ALP, uric acid and creatinine. Infected group controlled with

maduramicin showed a marked improvement in liver and kidney functions expressed by increasing the drop occurred in total serum protein, serum albumin, globulins and decreasing the elevation of serum activity of AST, ALT alkaline phosphatase, uric acid and creatinine lower than that of infected non treated group, these good results supported by *Berger et al., (1988)*. Infected group treated with diclazuril showed a significant difference by increasing total serum protein, serum albumin, globulins and decreasing serum AST, ALT, ALP, uric acid and creatinine to nearly reach to non infected control group and these findings similar to those obtained by *Hassan (2002)* who mention that treatment by diclazuril lead to great improvement in liver and kidney functions and what is proofed by *Hammoud (1998)* who evaluated the safety of diclazuril at their approved use levels and declared that it had no damaging effect on liver or other internal organs. Infected group treated with both drugs together reflected great improvement in general liver and kidney functions as mentioned in both drugs by decreasing tissue damage, liberating enzymes and loss of protein as a result of both dugs effectiveness. No significant difference was recorded in non infected treated groups with maduramicin, diclazuril or both drugs together than negative control group this result supported by *EL-Sayed (2002) and Hassan (2002)* demonstrated that no significant difference had been recorded in total serum protein, albumin, globulins, albumin globulin ratio, serum AST, ALT activities and serum uric acid level in non infected medicated groups with diclazuril as compared to negative control group.

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الملخص العربي

يعتبر مرض الكوكسيديا من أخطر الأمراض الطفيلية التي تصيب مزارع الدواجن وتعتبر الأيميريا تنيلا من أكثر أنواع الأيميريا ضراوة وتصيب حوالي 90% من إنتاج بداري التسمين. أجريت هذه الدراسة للتعرف على كفاءة عقار الماديوراميسين بجرعة 5 جزء في المليون، الدايكلازوريل بجرعة 2.5 جزء في المليون والاثنين معاً في الدجاج السليم والمعدى معملياً بجرعة 50.000 حويصلة من طفيل الأيميريا تنيلا لكل طائر عند عمر 15 يوم بعد ظهور الأعراض في اليوم الخامس بعد العدوى لمدة ثلاثة أيام متتالية من يوم 20 إلى يوم 22 ثم من يوم 30 إلى يوم 32 من العمر للحصول على أفضل الطرق للسيطرة على الكوكسيديا الأعرورية وتسجيل تأثير العلاج بكل منهما والتداخل بينهما على الفحص الكيميائي لمصل الدم. أوضحت النتائج أن العلاج بالداونين معاً لم يظهر أي تداخل سلبي بينهما وأن استخدامهما معاً أعطى أفضل النتائج بالتغلب على الأعراض الإكلينيكية للمرض وخفض معدلات الإصابة والوفيات وأعطت أقل عدد للبويضات وأفضل تحسن في وظائف الكبد والكلى مما يعكس التآزر بينهم دون أي آثار جانبية. كانت كفاءة الدايكلازوريل بدرجة أفضل من الماديوراميسين في تحسين الصفة التشريحية للأعورين وخفض عدد البويضات. المجموعات السليمة المعالجة لم يظهروا أي تغير معنوي مقارنة بالمجموعة الضابطة السلبية خلال مدة التجربة مما يعكس الاستخدام الآمن لكل منهما أو معاً.