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DRUG INTERACTION OF USING LINCOMYCIN HCL AND DICLAZURIL ALONE OR TOGETHER IN CONTROLLING CECAL COCCIDIOSIS IN CHICKENS.

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ABSTRACT

This study was carried out to test the interaction between lincomycin and diclazuril and its effect on experimentally infected chickens with E.tenella in order to evaluate any interference of lincomycin upon anticoccdeal efficacy of diclazuril and evaluate their efficacy on improving oocyst counting, hematological and biochemical parameters. It was observed that administration of diclazuril has potent anticoccideal efficacy with therapeutic recommended dose at (2.5 ppm) given after the symptoms had appeared (on 5th day of infection) for 3 successive days in drinking water. On the other hand lincomycin has a very weak anticoccideal efficacy at dose (30mg/kg.b.wt.) given after the symptoms of infection had appeared (on 5th day of infection) for 3 successive days in drinking water and didn't gave satisfactory benefits after the symptoms had appeared. Additionally, The use of lincomycin and diclazuril together did not show any antagonist interaction between them but had compatibilities between each other and that appeared in improving oocyst counting,, improved biochemical and hematological parameters.

INTRODUCTION

Coccidiosis is a protozoal disease of major economic importance in the poultry industry ***(Williams, 2005)***. Coccidiosis damages the gastrointestinal tract allowing for the birds to become more susceptible to other infectious diseases such as Salmonella spp., Histomonas meleagridis (Blackhead), or Clostridium perfringens which is linked with the development of necrotic enteritis ***(Chapman, 2009)***.

Amer et al., (2007) evaluated of the efficacy of water-soluble formulation of diclazuril (1%) in the prevention and control of mixed *Eimeria* infection in two experimental and natural field case of mixed *Eimeria* infection. Moreover, ***El-Banna et al., (2005)*** recorded that water soluble formulation of diclazuril induced a marked inhibitory effect on the different stages of the parasite life cycle in experimentally infected treated birds especially when applied on the day when blood first appeared in the faeces.

Hassan (2002) reported that diclazuril treated groups showed non significant changes in the hemograme or leucogram as compared to control throughout the experimental period and also demonstrated that non significant difference had been recorded in total serum protein, albumin, globulin, albumin globulin ratio, serum ALT, AST activities and serum uric acid level in non infected medicated groups with diclazuril as compared to control group.

Lincomycin is an antibiotic produced by *Streptomyces linconensis*. It belongs to the class of lincosamides, which are derevatives of an amino acid and a sulfur containing galactosides. Other substances belonging to the lincosamides group are clindamycin and pirlimycin.

Lincomycin is used as oral preparation in feed and water in poultry for treatment of bacterial enteric infection and as a performance enhancer in pigs. It is used for treatment and control of bacterial enteric, mycoplasmal respiratory infection, in infectious arthritis and as a performance enhancer ***(Barbara Rostel et al., 2003)***. Additionallly, ***The united states pharmacopeial convention (2008)*** cited that lincomycin has been shown to have efficacy against *Erysipelothrix insidiosa, Leptospira pomona, Mycoplasma species, Staphylococcus species, and Streptococcus species (except Streptococcus faecalis).* The activity of lincomycin against obligate anaerobes is seldom addressed in published literature; one exception is in vitro activity against *Fusobacterium necrophorum*. It also has activity against necrotic enteritis in chickens caused by susceptible organisms, such as *Clostridium perfringens*.

Wheelhouse et al., (1985) reported that depressions in body weight were accompanied by reductions in feed intake, although this effect was only significant for the birds fed lincomycin alone at 28 days relative to all other treatments in a floor pen trial using an in-feed coccidia model used to determine any interference of lincomycin upon salinomycin efficacy in broiler chickens.

Lanckriet et al. (2010) cleared that treatment with lincomycin completely stopped the development of necrotic lesions when drinking water medication with the antibiotics was evaluated as curative treatment in experimental model that uses coccidia as a predisposing factor within presence of coccidiostatics.

This work was conducted to investigate the interaction between lincomycin and diclazuril and its effect on experimentally infected chickens with *E.tenella*.

MATERIAL AND METHODS

A- Diclazuril (Diclosol):

It is a synthetic anticoccideal drug developed and described by Pharma Swede Co., Egypt. Diclosol is recommended at alevel of 2.5 ppm.

B- Lincomycin ((Linconeer):

It belongs to the class of lincosamides developed and described by Pioneer Pharma, Egypt. Linconeer is recommended at 30mg/kg.b.wt.

Experimental design:

In the present work one hundred mixed sex one day old Cobb strain chicks were divided into 5 main equal groups . All chicks were fed on ordinary ration free from any anticoccidial drug all over the experiment (42 days). *The first group* was kept non infected, non treated. *The second group* was infected with *E.tenella* and considered as positive non treated control group. *The third group* was infected with *E.tenella* and treated with lincomycin 30 mg/kg.b.wt.*for 3 successive days (day 19 to day 2). The fourth group was infected with E.tenella* and treated with diclazuril 2.5 ppm *for 3 successive days (day 19 to day 21). The fifth group was infected with E.tenella* and treated with both diclazuril and lincomycin, *for 3 successive days (day 19 to day 21).*

All groups were infected with *E.tenella* (50.000 sporulated oocyst/bird), except the 1st group kept non infected. At 14 days of age, fecal samples were collected daily from the 5th day after infection (day 18 of age) until 2weeks (day31 age). Two blood samples were collected (3 bird / group) on 21st, 28th, 35th, and 42nd day of age.

Laboratory examinations:

The oocyst count was carried out according to the method described by ***Abd El-Rahman et al., (1982)*** using the Mc Master counting chamber. Total erythrocytic and leucocytic counts were done according to ***Natt and Herric, (1952)***. Serum ALT, and AST activities were measured according to ***Reitman and Frankel, (1957),*** Creatinine according to ***Henry, (1974)*** and uric acid by the method of ***Barham and Trinder, (1972).*** Determination of total protein was performed according to ***Doumans et al.(1981).*** Electrophoretic analysis of chicken sera was done as recorded by ***Davis (1964) and Ornstein (1964)***.

Statistical analysis:

Data were statistically analyzed using one-way analysis of variance for comparison of mean of values of the various groups at a significant level of P▪ 0.05 and computerized using **SPSS 16.00.** ***(Petrie and Watson, 1999)***.

RESULTS AND DESCUSSION

The obtained data presented in tables from (1) to (8) showed that the infected group treated with lincomycin showed a significant increase in oocyst count and significant decrease in R.B.Cs count, significant increase in total leucocytic count, a significant increase in serum activity of ALT, AST, creatinine and serum uric acid and a significant decrease in total serum protein, serum albumin, globulins in comparison with non infected non treated group and other treated groups. Significant improvement in the measured oocyst count and hematological parameters were recorded in infected chickens treated with diclazuril and both drugs together appeared as increase in the RBCs count, significant decrease in serum activity of ALT, AST, creatinine and serum uric acid and a significant increase in total serum protein, serum albumin and globulins.

Table (1): The effect of lincomycin, diclazuril and both drugs together on oocyst count ($x10^3$)/gm feaces post infection day.□X ±SE n=3. P▪ 0.05.

	G2	G3	G4	G5
18 day	142.42 ±32.93	145.73 ±21.81	151.62 ±18.42	149.32 ±24.46
19 day	257.07 ±19.50	246.89 ±20.541	253.32 ±23.32	246.84 22.03±
20 day	304.09 ±32.196	311.87 ±21.84	295.20 ±17.60	279.60 ±12.55
21 day	515.52 ±14.63	493.33 ±30.70	177.30 ±8.838bd	181.24 ±18.027be
22 day	335.12 ±10.42	281.89 ±13.82	146.37 ±17.04bd	142.13 ±11.59be
23 day	255.09 ±24.992	205.61 ±8.241b	105.53 ±4.382bd	103.83 ±2.948be
24 day	182.87 ±9.368	95.57 ±2.243b	52.24 ±1.63bd	52.876 ±1.098be
25 day	81.45 ±9.851	32.65 ±2.341b	22.90 ±1.043b	21.26 ±1.167b
26 day	55.02 ±0.711	17.85 ±0.228b	11.74 ±0.157bd	9.55 ±0.369bef
27 day	19.91 ±0.041	9.34 ±0.245b	4.02 ±0.524bd	3.016 ±0.145bef
28 day	7.796 ±0.143	3.94 ±1.156b	2.946 ±1.155b	2.34 ±0.330b
29 day	3.583 ±0.181	1.876 ±0.564	1.806 ±0.608b	1.473 ±0.784b
30 day	1.783 ±0.075	1.28 ±0.151	0.893 ±0.063b	0.943 ±0.673b
31 day	1.53 ±0.754	0.81 ±0.136	0.00 ±0.00b	0.00 ±0.00b

N.B: Parameters with different letters are significant at P▪ 0.05.

Table (2): The effect of lincomycin , diclazuril and both drugs together on red blood corpuscles (R.B.Cs) $x10^6$ / mm^3post infection day.□X ±SE n=3. P▪ 0.05.

	G1	G2	G3	G4	G5
day 21	2.72 ±0.09	1.95 ±0.06c	1.97 ±0.07a	2.05 ±0.08a	2.07 ±0.025a
day 28	2.79 ±0.26	1.99 ±0.18c	2.06 ±0.04a	2.18 ±0.07abd	2.23 ±0.13abe
day 35	2.79 ±0.06	2.02 ±0.02c	2.27 ±0.25a	2.53 ±0.21bd	2.6 ±0.056be
day 42	2.98 ±0.04	2.23 ±0.09c	2.54 ±0.16a	2.76 ±0.13b	2.95 ±0.23bf

N.B: Parameters with different letters are significant at P▪ 0.05.

Table (3): The effect of lincomycin , diclazuril and both drugs together on white blood corpuscles(W.B.Cs)x10^3 /µl post infection day.□X ±SE n=3. P▪ 0.05.

	G1	G2	G3	G4	G5
day 21	20.12 ±0.61	25.52 ±0.31c	24.91 ±0.51a	22.11 ±0.06abd	22.14 ±0.09abe
day 28	20.82 ±0.58	23.82 ±0.97c	23.09 ±0.10a	21.85 ±0.19abd	21.55 ±0.32be
day 35	21.67 ±0.44	22.99 ±0.11c	22.81 ±0.37a	21.48 ±0.20bd	21.82 ±0.43b
day 42	21.68 ±0.34	22.7 ±0.72c	22.47 ±0.39a	21.2 ±0.11bd	21.27 ±0.12be

N.B: Parameters with different letters are significant at P▪ 0.05.

Table (4): The effect of lincomycin , diclazuril and both drugs together on Alanine aminotransferase (ALT U/L) post infection day.□X ±SE n=3. P▪ 0.05.

	G1	G2	G3	G4	G5
day 21	47.48 ±0.86	93.63 ±1.08c	88.36 ±1.28ab	80.96 ±0.57abd	78.27 ±1.20abe
day 28	44.92 ±0.61	88.27 ±1.18c	77.52 ±6.24ab	57.6 ±0.88abd	54.1 ±0.94abe
day 35	42.78 ±0.47	74.1 ±0.77c	66.12 ±1.97ab	52.48 ±0.28abd	48.89 ±0.57abef
day 42	43.92 ±0.53	67.84 ±0.56c	58.63 ±1.43ab	51.51 ±0.37abd	49.55 ±0.28abe

N.B: Parameters with different letters are significant at P▪ 0.05.

Table (5): The effect of lincomycin , diclazuril and both drugs together on aspartate aminotransferase (AST U/L) post infection day.□ X ±SE n=3. P▪ 0.05.

	G1	G2	G3	G4	G5
day 21	42.81 ±0.56	86.63 ±1.082c	82.03 ±3.79a	73.62 ±4.18abd	68.27 ±1.20abe
day 28	44.78 ±1.81	78.27 ±1.18c	71.19 ±0.61a	64.26 ±3.28ab	58.76 ±3.30abe
day 35	43.78 ±0.65	68.77 ±1.65c	59.79 ±2.56ab	53.82 ±2.19abd	48.89 ±0.57be
day 42	42.59 ±0.85	67.84 ±0.56c	53.95 ±2.04ab	51.18 ±0.40ab	44.55 ±1.12bef

N.B: Parameters with different letters are significant at P▪ 0.05.

Table (6): The effect of lincomycin, diclazuril and both drugs together on serum creatinine (mg/dl) post infection day. X ±SE n=3. P 0.05.

	G1	G2	G3	G4	G5
day 21	0.81 ±0.12	1.52 ±0.11c	1.06 ±0.28a	0.96 ±0.14b	0.95 ±0.2b
day 28	0.75 ±0.01	1.22 ±0.12c	0.93 ±0.32ab	0.78 ±0.16bd	0.77 ±0.09be
day 35	0.73 ±0.01	1.09 ±0.02c	0.86 ±0.01ab	0.76 ±0.02bd	0.74 ±0.02be
day 42	0.76 ±0.02	0.95 ±0.01c	0.77 ±0.01b	0.73 ±0.01b	0.75 ±0.02b

N.B: Parameters with different letters are significant at P 0.05.

Table (7): The effect of lincomycin, diclazuril and both drugs together on serum uric acid (mg /dl) post infection day. X ±SE n=3. P 0.05.

	G1	G2	G3	G4	G5
day 21	6.68 ±0.26	9.2 ±0.21c	8.56 ±0.23ab	7.95 ±0.04abd	7.17 ±0.12bef
day 28	6.23 ±0.06	8.86 ±0.04c	7.73 ±0.11ab	7.29 ±0.33ab	7.33 ±0.22ab
day 35	5.82 ±0.13	8.32 ±0.11c	7.21 ±0.12ab	6.95 ±0.02ab	6.61 ±0.15abe
day 42	5.32 ±0.07	7.52 ±0.21c	6.69 ±0.09ab	5.72 ±0.06abd	5.84 ±0.09be

N.B: Parameters with different letters are significant at P 0.05.

Table (8): The effect of lincomycin , diclazuril and both drugs together on total serum protein (g /dl) post infection day. X ±SE n=3. P 0.05.

	G1	G2	G3	G4	G5
day 21	4.3 ±0.32	2.9 ±0.2c	3.1 ±0.60a	3.9 ±0.5abd	3.8 ±0.65abe
day 28	3.23 ±0.07	2.65 ±0.41c	2.84 ±0.06a	3.06 ±0.13abd	3.43 ±0.32bef
day 35	3.6 ±0.03	2.83 ±0.07c	3.13 ±0.33ab	3.37 ±0.19abd	3.67 ±0.03bef
day 42	3.57 ±0.04	2.96 ±0.13c	3.3 ±0.2ab	3.76 ±0.03bd	3.65 ±0.18bc

N.B: Parameters with different letters are significant at P 0.05.

Table (9): The effect of lincomycin, diclazuril and both drugs together on serum protein fractions (g /dl) at end of experiment. X ±SE n=3.P▪ 0.05.

	G1	G2	G3	G4	G5
Pre albumin	0.319 ±0.104	0.138 ±0.006c	0.190 ±0.037a	0.321 ±0.027bd	0.211 ±0.027be
Albumin	0.890 ±0.051	0.823 ±0.033c	0.821 ±0.008a	0.854 ±0.100abd	0.943 ±0.080bef
Total albumin	1.259 ±0.026	0.993 ±0.031c	1.115 ±0.048b	1.165 ±0.019bd	1.234 0.035bef
α1 globulin	0.535 ±0.035	0.521 ±0.025	0.616 ±0.087ab	0.797 ±0.013abd	0.531 ±0.009
α2 globulin	0.298 ±0.038	0.203 ±0.013c	0.185 ±0.030ab	0.187 ±0.005ab	0.365 ±0.048abef
Total α globulin	0.833 ±0.015	0.724 ±0.019c	0.801 ±0.028	0.986 ±0.009bd	0.896 ±0.019be
β1 globulin	0.254 ±0.021	0.260 ±0.027	0.226 ±0.018	0.302 ±0.007abd	0.264 ±0.008f
β2 globulin	0.279 ±0.014	0.236 ±0.005	0.263 ±0.025	0.275 ±0.003	0.269 ±0.014
Total β globulin	0.533 ±0.017	0.496 ±0.016	0.489 ±0.021	0.577 ±0.004	0.533 ±0.011
γ1 Globulin	0.597 ±0.018	0.500 ±0.020c	0.540 ±0.025a	0.788 ±0.090bd	0.607 ±0.004be
γ2a Globulin	0.295 ±0.037	0.250 ±0.007c	0.306 ±0.037b	0.310 ±0.006b	0.320 ±0.009b
γ2b Globulin	0.108 ±0.066	0.036 ±0.003c	0.053 ±0.006a	0.063 ±0.014	0.144 ±0.059bef
Total γ globulin	1.01 ±0.142	0.786 ±0.134c	0.899 ±0.141a	1.161 ±0.232bd	1.071 ±0.134be

N.B: Parameters with different letters are significant at P▪ 0.05.

In the light of the present findings, it could be stated that in infected group treated with lincomycin, a significant elevation in oocyst count and this result agreed with ***FDA (1989)*** which reported in pen trial study, that there is no interference of lincomycin on the anticoccideal efficacy of halofuginone hydrobromide but when comparing the infected non treated group with infected group treated with lincomycin (4 gm/ton) showed no differences. A significant decrease in hematological parameters in the RBCs count and a significant increase in serum ALT,

AST, creatinine and uric acid activity in comparison with infected treated group with diclazuril or both drugs together was recorded. There is non significant drop of total serum protein, serum albmin, globulins which resulted due to the infection. A slight decrease in serum ALT, AST, creatinine and uric acid in comparison with non infected non treated group was recorded.

The infected group treated by diclazuril showed an improvement in oocyst count and the hematological parameters by elevating the RBCs count. These result similar to what was reported by ***El-Sayed, (2002) and Hassan, (2002)***. The results showed a significant difference by increasing the drop of total serum protein, serum albumin and globulins which resulted due to the infection. A decrease in serum ALT, AST, creatinine and uric acid nearly to non infected control group were recorded.These findings are similar to those obtained by ***Hassan (2002) and El-sherbeny E.M. (2011).*** They mentioned that treatment by diclazuril in infected chickens with coccidia lead to great improvement in liver and kidney functions and what is prooved 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, as indicated in the biochemical analysis of blood total protein, albumin, glucose, creatinine and some liver enzymes.

In infected group treated with both drugs lincomycin and diclazuril revealed a significant difference in oocyst count and hematological parameters than infected non treated group. That was apparent by an increase in RBCs count, improvement in general liver and kidney functions as mentioned by decreasing tissue damage which liberates enzymes and the loss of protein as a result of both drugs given concomitantly together.

CONCLUSION

Lincomycin does not affect the anti coccidial efficacy of diclazuril and the use of lincomycin and diclazuril together did not show any antagonistic interaction between each other.

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التداخل الدوائي للإستخدام المتزامن لكل من اللينكومايسين هيدروكلوريد والدايكلازوريل في السيطرة علي الكوكسيديا الأعورية في الدجاج

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استهدف هذا البحث دراسة فاعلية دواء اللينكومايسين و الدايكلازويل ودراسة مدى التداخل بينهم فى الدجاج المعدي معمليا بالأيميريا تنيلا، ودراسة مدي تأثير دواء اللينكومايسين على كفاءة الدايكلازويل في علاج الكوكسيديا ودراسة مدي تأثيرهم علي تحسن كل من صورة الدم و الفحص الكيميائي لمصل الدم. وقد لوحظ أنه بإستخدام عقار الداي كلازوريل أثبت أن له تأثير علاجي قوي كمضاد للكوكسيديا الأعورية وأكثرها فاعلية بجرعة (2.5 جزء في المليون) بعد ظهور الأعراض (اليوم الخامس بعد العدوي) لمدة ثلاثة أيام بدرجة أفضل من اللينكومايسين الذي لم يعطي نتائج مرجوة في مكافحة الكوكسيديا الأعورية.

علي صعيد أخر، أثبت العلاج بالدوائين معا أنه لم يظهر أي تداخل سلبي بينهما وأن استخدامهما معا أعطي أفضل النتائج وقد انعكس هذا علي تحسن تعداد البويضات و صورة الدم و وظائف الكبد والكلي وكذلك بروتينات الدم.