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The Injection Effect of Local Oily Cadmium Ligand Compound on the Blood Performances of Layer Chickens

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Abstract: An oily injectable administration of [Di nitrate- bis {p-methyl anilino phenyl aceto nitrite} cadmium (II)] -2H_2O and its molecular formula [Cd $(HL)_1(NO_3)_2$] - 2H_2O and tested against *E. coli* (sensitivity test) *in vitro* showed inhibitory zone of 12 mm. The CLC was injected subcutaneously in the layer chickens neck (*Lohman brown*) at different levels, T_1 0.0%, T_2 0.25%, T_3 0.50% and T_4 1.0%. After five days *E. coli* was orally administrated by drinking water at concentration of 8500 x 10^6 cells/ml (highly pathogenic) as causative agent of Salpengitis and Ovaritis. The results after four months were revealed that the treated chickens showed a resistance against *E. coli* infection; moreover, all the blood parameters are within the normal ranges. The protective effects of CLC in respect to blood biochemical's activities which are then confirmed by measuring the residual concentration of Cd in liver and egg of treated chickens by using the graphite furnace atomic absorption. The results showed that, the liver and eggs are free from any residues. The hematological results of this study are associated with the normal control (T_1), while there are significant increases in blood parameters of (T_4) in Hb, PCV, RBCs, WBCs, Glucose, Total protein, Total albumin, Total globulins and High Density Lipoprotein (HDL), but there was significant decrease in Low Density Lipoprotein (LDL).The obtained results refer to the safety use of CLC as a synthetic chemical prophylactic agent against *E. coli* during the production period of layers, in addition to its inexpensive cost.

Key words: Cadmium ligand compound (CLC), blood of layers, prophylaxis, against *E. coli*

INTRODUCTION

CLC is a yellowish powder, related to organic aminonitril compounds (Rawaa and Kh., 2010), which are well known as biologically active compounds, used in many pharmaceutical preparations (Perrier *et al*., 2000). The aminonitrile compound is used to explain the biological reactions, by acting as biological inhibitors and chelating agents (Postel *et al*., 2000). CLC was identified by I.R spectroscopy and determined by atomic absorption and U.V visible spectrophotometers (Rawaa and Kh., 2010). The magnetic susceptibility and electrical conductivity of CLC were also measured (Rawaa and Kh., 2010). Cadmium (Cd) is a heavy metal, known to be highly toxic to both human and animal. Cd is widely distributed in the environment due to its various industrial uses, some of the toxic effects of Cd (renal dysfunctions, hepatic damage and anemia) due to of its interference with ions metabolism (Nordberg *et al*., 1985). Cd causing oxidative damage in different tissues by enhancing the per oxidation of membranous lipids in the tissues and altering the cells antioxidant system by its interaction with the cell organelles (Sarkar *et al*., 1995). The synthesis of Cd compounds as ligand (an operation that protect tissues from deleterious effect of free radicals and protect liver from toxicity when Cd used) (Vaidya *et al*., 1996). The administration of Cd disrupted the metabolism of Ca, Fe, Cu, Zn, Se ions and decrease their levels in the organism (Jeyaprakash and Chinnaswamy, 2005). The present work is studying the CLC toxicity and its protective role against *E. coli* in layers.

MATERIALS AND METHODS

The present study has designed to use the CLC as antibacterial agent in layers. CLC has been produced for first time in Basic sciences section, College of Agriculture, university of Baghdad, it is a [Di nitrate- bis {p-methyl anilino phenyl aceto nitrite} cadmium (II)] -2H_2O, with a chemical formula [Cd $(HL)_1(NO_3)_2$]-2H_2O (1). Cd as a complex has a biological activity against a number of microorganisms. The CLC is of 363°C melting points, 716 molecular weight and 17.31% Cd percent in complex (Rawaa and Kh., 2010). The oily material was used to dilute CLC to various concentrations 0.25%, 0.5% and 1.0%, were prepared in the department of bacteriology, at Al-Kindy Company, as following:

Liquid paraffin (90) ml; Arlacil (9) ml; Twin80 (1) ml: 48 healthy layer chickens (*Lohman brown)*, of 49 weeks age, weighing 1800-2200 gm, have been used in this study. All birds were housed in an optimal Poultry Field

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Table 1: The composition of the experimental diet

Ingredient	%
Yellow corn	30.80
Wheat	37.00
Soya bean (48%)	18.00
Concentrated animal protein (40%)	5.00
Vitamins and minerals	0.10
Soya bean oil	0.30
DCP stone	7.50
NaCl	0.03
DCP (18%)	1.00

Mathematical chemical analysis of diet ingredients according to (NRC, 1994) was (Metabolized energy: 2752 K calorie\K.; mathematical crude protein: 17.50%; lycine: 1.10%; methionine: 0.41%; Methionine + Cystine: 0.75%; calcium: 3.40%; available phosphor: 0.42%; linolenic acid: 1.05%

of the Animal resource Department in College of Agriculture, University of Baghdad during the period of May 1 to August 30, 2011. They were fed on crushed diet with a formula shows in Table 1. The chickens were divided into four main groups (T_1, T_2, T_3 and T_4), each group divided into 3 subgroups and injected subcutaneously in the neck by CLC dose of 0.25%, 0.50% and 1.0% respectively, uninjected T_1 was taken as control.

The (*Escherichia coli*) was obtained by the assistant of laboratory of microbiology Department, College of Veterinary Medicine, University of Baghdad. The *E. coli as* salpengitis and Ovaritis agent has been given to the layers T_2, T_3 and T_4 by the drinking water at 8.500×10^6 cell/ml concentration. The clinical observations were recorded in all groups during the period of four months.

Hematological analysis for the collected blood samples of birds (62 wks.) was done in August 10, 2011 by using EDTA tubes to estimate Hb, PCV. RBC and WBC counts (Coles, 1974), other samples were collected without EDTA, to measure the blood sugar(Coles, 1974), total triglyceride (Toro and Ackermann, 1975), cholesterol (Shih *et al*., 2000), High Density Lipoprotein (HDL), Low Density Lipo-protein (LDL) (Warnick *et al*., 1995), total protein (Henry *et al*., 1974), Total albumin (Doumas and Biggs, 1972) and total globulins (Al-Omary, 2001). The blood samples were analyzed by using (Bio-MaizFrance) Spectrophotometer.

To determine the Cd residues in chickens bodies, the samples of liver and eggs were collected after 10, 30, 85 days of CLC injection. The specimens ashed at 600°C for 48 hr. The ash was dissolved in few drops of an acidic mixture containing nitric acid and perochloric acid (1:3) and diluted to concentration of 20 ppm by using deionized distal water (Vogal, 1972; Ahmed and Mayouf, 2009). The Cd concentration were measured by graphit furnace atomic absorption by the assisting of serving Lab., Dept. of Chemistry, College of Science. Results were statistically analyzed by using Complete Randomized Design (CRD) and SAS (2001) programme. The Duncans Multiple Range Test (DMRt) was used to show the less significant differences LSD between means of experimental groups at ($p<0.05$) probability level (Duncan, 1955).

RESULTS AND DISCUSSION

Initial test was done invitro by using CLC against *E. coli* and it was found that the inhibitory zone was 12 mm.

Table 2 revealed that the T_4 gave normal ranges with significant increasing in Hb, PCV, RBC and WBC counts as compared with the control (T_1), while, Jeyaprakash and Chinnaswamy (2005) mentioned that Cadmium chloride inhibit the absorption of Fe and Cu and utilization in rats. Total WBC count in CLC treated chickens showed no significant differences as compared with the control (T_1). Which means that, CLC protect the chickens from induced infection by *E. coli*, thus prevent salpengitis and ovaritis in laying chickens. Normally the WBC was increase 9000-12000 cell/ml during the infection because the WBC is an indicator for any pathological status in chickens. Pirarat *et al*. (2008) declared that B-lymphocytes production was increased in the spleen of Tilpia fish (*Oreochromis niloticus)*, when the fish were subjected to the higher dose of Cd.

The results in Table 3 showed significant decreasings in glucose levels in T_4 and it were associated with T_1 (control). That means CLC does not change the secreted insulin level which inturn doesn't affect the pancreases' function. The insulin plays an important role in the metabolism of carbohydrates and maintains the glucose level in blood. Khosla *et al*. (1995) declared that the insulin will increase the glucose oxidation and liberate energy in the cells. In liver the insulin converted the glucose to glycogen by the Gluconeogenesis process, (Gluconeogenesis: some amino acids were converted to sugar or fatty acids in the body).

Total protein, albumin and globulins were in a normal range associated with the normal (T_1), but there is a significant increasing in T_4, this result will support the birds immunity. Coles (1986) mentioned that albumin is the major plasma protein in poultry and all blood

Table 2: The effect of CLC on hematological parameters

	Experimental groups			
Parameters	T_1 (0.0%)	T_2 (0.25%)	T_3 (0.50%)	T_4 (1.0%)
Hb gr./100 ml	7.80d±0.12	9.1c±0.58	10.4b±0.17	11.3a±0.17
PCV % (v\v)	24.00d±1.15	28.0c±1.15	32.0b±1.73	35.0a±0.15
WBC cell/ml	5.30a±0.04	5.4a±0.04	5.2a±0.06	5.5a±0.04
RBC cell/ml	3.09c±0.05	2.5d±0.06	3.7b±0.02	4.8a±0.12

T_1: (0.0%), T_2 (0.25%), T_3 (0.50%), T_4 (1.0%). Small letters in the table refer to the presence of significant differences between the groups

Table 3: The effect of CLC on blood glucose and protein levels

	Experimental groups			
Parameters mg/100 ml	T_1 (0.0%)	T_2 (0.25%)	T_3 (0.50%)	T_4 (1.0%)
Glucose	223.00b±13.2	245.00a±0.40	228.00b±4.6	222.00b±4.60
Total protein	3.60a±0.50	3.20b±0.29	3.40a±1.5	3.60a±0.40
Albumin	2.23b±0.13	2.75a±0.14	2.64a±0.12	2.97a±0.02
Total globulins	2.26b±0.15	2.48b±0.12	2.70a±0.12	2.89a±0.12

T_1: (0.0%), T_2(0.25%), T_3(0.50%), T_4(1.0%). Small letters in the table refer to the presence of significant differences between the groups

Table 4: The effect of CLC on blood lipids

	Experimental groups			
Parameters mg/100 ml	T_1 (0.0%)	T_2 (0.25%)	T_3 (0.50%)	T_4 (1.0%)
Total cholesterol	99.0a±2.80	82.0b±1.10	93.0b±2.3	97.0a±7.13
Total triglycerides	128.5a±8.60	86.2b±11.5	89.0b±5.7	122.9a±16.7
HDL	152.0a±1.15	142.0b±1.50	144.2b±0.5	151.0a±1.70
LDL	298.0a±1.50	240.0c±1.50	216.0d±0.5	252.0b±1.70

T_1: (0.0%), T_2(0.25%), T_3(0.50%), T_4(1.0%). Small letters in the table refer to the presence of significant differences between the groups

proteins are in constant values in blood. Heafele *et al.* (1997) recorded that, both, total protein and globulins were affected the total albumin, because the insulin decreased the deaminocarboxylation of amino acids in chick's body. Deaton *et al.* (1969) found that, any environmental pathological or dietary changes will affect liver function and lymphocytes, which inturn change the blood protein levels. Deaton *et al.* (1969) also reported that liver and lymphocytes are major sources for Immunoglobulin's (Igs) production which are responsible of bird immunity.

Table 4 showed that cholesterol was associated with the normal range as compared with T_1, there was a significant increasing in T_4 that which means the CLC doesn't interfere with cholesterol metabolism. Studies carried out by Pransa (2000) mentioned that perfect absorption and metabolism of cholesterol and bile acids obtained by allowing them to stay for a long time in digestion tract. Triglycerides also were within the normal ranges with control (T_1) and showing significant increasing in T_4. This result explains that, the CLC has no toxic effect on the liver and not affected the triglycerides synthesis. Scott *et al.* (1982) mentioned that triglycerides will bind to cholesterol in the liver to form the lipo-protein molecules. HDL and LDL (Table 4) showed normal range as compare with T_1, but there was a significant increasing in T_4, a result that preserve birds healthy status. Crouse (1985) reported that LDL is very important, because it is the main factor for transportation of the cholesterol, triglyceride and phospholipids fat between the blood and cells. The decreasing of LDL caused accumulation of both cholesterol and triglyceride in blood (Lewington, 2007). All results referred that T_4 showed best results than T_2 and T_3 as compared to control, because the pharmaceutical product gave best results without any side effect. Sharp increasing or decreasing than normal ranges in blood parameters mean that CLC at 1.0% concentration preserve the normal ranges of blood parameters, as showing in Table 2-4.

Table 5: The cadmium concentration in ashed liver and eggs

Samples	Period of collection after injection/day	Samples numbers	Cadmium µg/ml
Egg	10	18	-0.027
Egg	30	18	0.006
Egg	85	18	-0.027
Liver	30	18	-0.027
Liver	85	18	-0.029

Results from Table 5 showed that ashed livers and eggs were empty from any Cd residues, which in turn very important for human health. CLC is a cheap and available drug material that 1 gm of 1.35$ cost is enough for 100 birds (Daived *et al.*, 1988-1989).

The results of the present study indicate that CLC was effective to protect layers from induced *E. coli* infection and 1.0% concentration is best to be used as a synthetic chemical prophylactic antibiotic in layers.

Abbreviations: CLC: [Dinitrato- bis {p-methyl anilino phenyl aceto nitrite} cadmium (II)]. $(HL)_1$: {p-methyl anilino phenyl aceto nitrite}. Cd: Cadmium. *E. coli*: *Escherichia coli*. T_1, T_2, T_3 and T_4: are representing experimental chicken groups. Hb: Hemoglobin. PCV: Packed Cell Volume. RBCs and WBCs: Red and White Blood Cells.

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