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A Comparative Histological Study Between Injection and Oral Administration of New Cadmium Aminonitril Complex (CAC) in Layer Chickens to Control or Treat *E. coli* Infection and Their Counts in Digestive Tract

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Abstract: Cadmium of alpha-aminonitrile Complex (CAC) is a [Di nitrato-bis {(p-methyl anilino) phenyl acetonitrile} Cadmium (II)]. (2)Hydrate, $[Cd(HL)_2(NO_3)_2].2H_2O$ tested against *E. coli* (sensitivity test) *in vitro* showed inhibitory zone of 12 mm. 90 healthy layer chickens (*Lohman brown)*, of 49 weeks age, weighing 1800-2200 gm, have been used in this study. They are divided into ten main groups, five groups for each experiment (T_1, T_2, T_3, T_4 and T_5), each main group divided into 3 subgroups. All birds were housed in an optimal poultry field. In experiment 1, the CAC was injected subcutaneously into the layer chicken at different levels, (T_2 0.25%, T_3 0.50% and T_4 1.0%). After five days *E. coli* was orally administrated by drinking water for the treatments (T_2, T_3, T_4 and T_5) at titer of 8500 x 10^6 cells/ml (highly pathogenic) as causative agent of Salpingitis and Ovaritis. Uninjected uninfected T_1 was used as control. In experiment 2, *E. coli* was orally administrated to T_2, T_3, T_4 and T_5 by drinking water at titer of 8500 x 10^6 cells/ml (highly pathogenic) as causative agent of Salpingitis and Ovaritis. After two days the CAC has been used to treat the layer chickens at different levels, (T_2 0.25%, T_3 0.50% and T_4 1.0%) by mixing it with the diet. Uninfected untreated T_1 0.0% was used as a control. T_5 was important to identify the infection and the count of *E. coli* infection and its count in infected uninjected chickens. The results after four months showed a resistance against *E. coli* infection by the treated chicken in both experiment. The results were confirmed by measuring the residual concentration of Cd (II) ions in liver and egg samples of treated chicken by using the atomic absorption. The histopathological results revealed that histological structure is normal in all specimens of (liver, kidney, muscle, oviduct and cerebellum), which is associated with the control (T_1). The specimens of liver and kidney of T_5 at 8500 x 10^6 cells/ml of *E. coli* concentration showed pathological changes. Liver showed amyloidosis, congestion of central vein with RBC, increased in coffer cells. Kidneys represent congestion in interstitial tissue and distended of bowman's space. The liver and egg specimens of treated chicken showed that they were free from any Cd (II) ion residues. *E. coli* counts in both experiments showed decreasing in *E. coli* count and increasing of CAC concentrations. The obtained results refer to the safety use of CAC as a synthetic chemical prophylactic or treating agent against *E. coli* during the production period of layers, in addition to its inexpensive cost.

Key words: Cadmium aminonitrile Complex (CAC), comparative histology, layers, against *E. coli*

INTRODUCTION

The alpha-aminonitrile compounds had a biological activity, by their acting as biological inhibitors and chelating agents for pathogens (Njoroge *et al*., 1996). Beside that an inorganic complexes have been used in many pharmaceutical industries as chemotherapeutic agent against infectious diseases (Hidetoyo *et al*., 2002). Jeyaprakash and Chinnaswamy (2005) described the biologically active compounds, which they have antioxidant properties contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. The heavy metals complexes have been recently used and Cd is one of them. Cd constituents from natural resources (Chowdhury and Islam, 2004; Parihar *et al*., 2006) as well as synthetic (Daula *et al*., 2004). It is highly toxic metals, with no known beneficial physiologic role (Jarup *et al*., 1998; Anna *et al*., 2004). Yoshihide *et al*. (2008) mentioned that thyroid gland function of rats affected when treated with $CdCl_2$. Studies carried out by Jahan *et al*. (2006) proved that Cd (II) metal Cystine complex when synthesized, it has antibacterial and antifungal activities in brine shrimps. As a matter of fact no such agent till now can able to destroy effectively pathogenic microorganism. It is mainly because these pathogenic organisms are developing resistance heriditically towards these agents. It is therefore necessary to find out consistently new, more safe, effective and

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inexpensive agents for the purpose. In this subject, a few metal complexes has already been appeared in the literature (Sultana *et al*., 2003; Islam *et al*., 2002) as antibacterial agents. Also Treshchalina *et al*. (1979) studied antitumor properties of some amino acid complexes of copper. These studies above inspired us to study the antimicrobial activity of CAC compound. In addition to differentiate its cytotoxic effect between administrated it orally or as oily injectable preparation.

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 Kcalorie\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%

MATERIALS AND METHODS

The present study has designed to use the CAC as antibacterial agent in layers; it has been produced for first time in Basic sciences section, College of Agriculture, university of Baghdad, CAC is a [Di nitrato-bis {(p-methyl anilino) phenyl acetonitrile} Cadmium (II)](2)Hydrate, with a chemical formula [Cd $(HL)_2(NO_3)_2].2H_2O$, this compound was synthesized from alpha-aminonitrile with Cd (II) ion (Rawaa and Abd-Al-Hassan, 2010). It's a yellowish powder, melting at 263°C, its molecular weight 716 and Cd percent in complex was 17.31%. The structure of CAC was characterized by FT.IR spectroscopy, U.V visible spectrophotometer and atomic absorption; also the magnetic susceptibility and electrical conductivity of CAC were measured (Rawaa and Abd-Al-Hassan, 2010). 90 healthy layer chicken (*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 of the Animal resource Department in College of Agriculture, University of Baghdad during the period of May 1 to August 30, 2011. They divided into ten main groups (five groups for each experiment), then each main group divided into 3 subgroups. They were fed on crushed diet with a formula shows in Table 1. The (*Escherichia coli*) *as* Salpingitis and Ovaritis causative agent was obtained by the assistant of laboratory of microbiology Department, College of Veterinary Medicine, University of Baghdad.

Experiment 1: The oily material was used to dilute CAC 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.

45 chickens were divided into five main groups (T_1, T_2, T_3, T_4 and T_5), each main group divided into 3 subgroups, T_2, T_3, T_4 injected subcutaneously in the neck by CAC dose of 0.25%, 0.50% and 1.0% respectively, uninjected T_1 was used as control. T_5 was important only to identify *E. coli* infection in infected uninjected chickens. The (*Escherichia coli*) *as* Salpingitis and Ovaritis agent has been given to the layers T_2, T_3, T_4 and T_5 by the drinking water at 8.500 x 10^6 cell/ml concentration. The clinical observations were recorded in all groups during the period of four months.

Experiment 2: 45 chickens were divided into five main groups (T_1, T_2, T_3, T_4 and T_5), each group divided into 3 subgroups. After a week of chicken accumulization the (*Escherichia coli*) *as* Salpingitis and Ovaritis agent has been given to the layers T_2, T_3, T_4 and T_5 by the drinking water at 8.500 x 10^6 cell/ml concentration. The powder of CAC material was mix with diet for treating *E. coli* induced infection. After two days of infection the treatment began in various concentrations of CAC 0.25%, 0.5% and 1.0%, for T_2, T_3 and T_4 respectively. Untreated, uninfected T_1 was used as control.

In both experiments T_5 was important only to identify *E. coli* infection and its counts in direct smears from intestinal tract in infected uninjected and untreated chickens. At the end of experiments a chicken from each subgroup had been slaughtered to identify and estimate *E. coli* count from a direct smears of small intestinal tract (duodenum) by using the procedure of Harrigan and Mecance (1976), smears had been taken by autoclaved swabs then stepping into a nutrient broth then incubated at 37°C for 48 h. Untill it become turbid, shake it carefully to ensure equal suspension of *E. coli* and a procedure of William (2003) was followed to estimate *E. coli* count in each sample. Then *E. coli* had been cultured on Nutrient and MacConkey Red agar (differential agar of *E. coli*) to identify *E. coli* colonies, also it tested biochemically by (In dole and Methylene Red) tests (Carter, 1973).

Histopathological examination: After one month, then after three months a chicken from each sub group of both experiments were slaughtered. And small pieces of brain, oviduct, kidney, liver and skeletal muscle (pectoral muscle). They were fixed in 10% formalin solution, dehydrated with upgrading concentration of ethanol alcohol started with 50%-100%, embedded in paraffin, sectioned at (5 µm thickness) and stained with hematoxyline- eosin stain (Luna, 1968).

To determine the Cd residues in chicken bodies (Exp. 1 and 2), the samples of liver and eggs were collected after 10, 30, 85 days of CAC dosing. The specimens ashed at 600°C for 48 hr. Then the ash was gave to the

serving Lab., Dept. of Chemistry, College of Science to estimate Cd residues in the samples. Results of both experiments 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 *in vitro* by using CAC against *E. coli* and gave an inhibitory zone of 12 mm. Jahan *et al.* (2006) had proved that Cd (II) cystine has antibacterial and antifungal activity (sensitive test) against numbers of bacteria and fungi, for example (*Salmonella typhi*, 36 mm at 80 µg concentration and 24 mm at 50 µg concentration; *Staphylococcus aureus*, 35 mm at 80 µg concentration and 22 mm at 50 µg concentration; *Aspergillus niger*, 39 mm at 600 µg concentration and 33 mm at 300 µg concentration; *Aspergillus flavus*, 34 mm at 600 µg concentration and 28 mm at 300 µg concentration). The affected birds in T_5 occasionally were showing Pathological symptoms that revealed perihepatitis, pericarditis, ascitis, gaseous ovary, atrophied oviduct which are the main pathological symptoms of ovaritis and Salpengitis (Azusa *et al.*, 2007). *E. coli* cultured on nutrient agar by using samples collected from intestine of T_5, which gave a large mucoid, circular, convex, smooth, white to yellowish white and sometimes translucent colonies. While gave a pin pointed colonies in MacConkey Red agar (Coles, 1974). *E. coli* broth tested biochemically by Iodole test which gave a brilliant red colorring below in the test tube and Methylene Red test gave a distinct red color (Carter, 1973).

Vaidya *et al.* (1996) mentioned that biological compounds with antiperoxidative and antioxidant properties. Therefore Cd (II) ion synthesized as a complex an operation to give it antiperoxidative and antioxidant properties to protect liver from toxicity due to use heavy metal for treating bacterial pathogens. Because cadmium toxicity could be carried out into the body by zinc binding proteins which contain zinc finger protein (zinc and cadmium are in the same group on the periodic table, contain the same oxidative state (+2)) due to these similarities, cadmium can replace zinc and bind up to ten times more stronger than zinc and difficult to remove in certain biological systems (Report on carcinogens 11th Edn.; Lane and Morel (2000); Friberg (1983)).

Experiment 1: The histopathological results of present study in all sub groups of T_2, T_3 and T_4 which treated by injection of CAC in infected chicken with 0.25% and 0.5% and 1.0% respectively, showed no pathological changes in all examined specimens of liver, oviduct, muscles and brain (cerebellum) (Fig. 1-4). That disagreed with

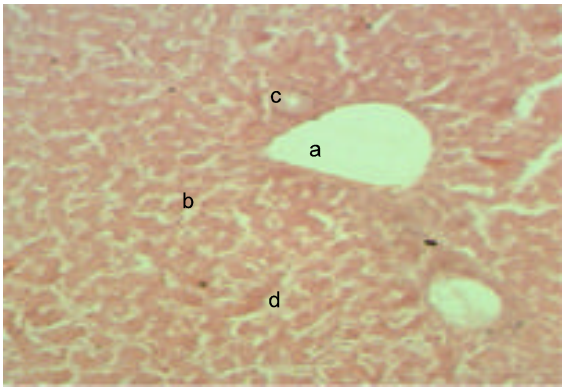

Fig. 1: Histological section of liver showing: (a) Central vein, (b) Cord of hepatocytes, (c) Bile duct, (d) Hepatic sinusoid (H&E) stain 200x

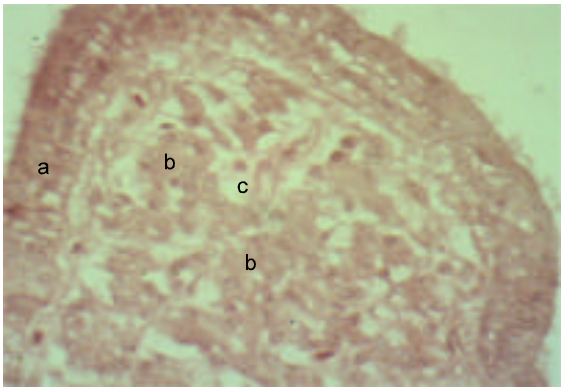

Fig. 2: Histological section in mucosal fold of oviduct (magnum) showing: (a) Normal epithelium lining, (b) Serous acini of glands, (c) Lamina properia, (H&E) stain 200x

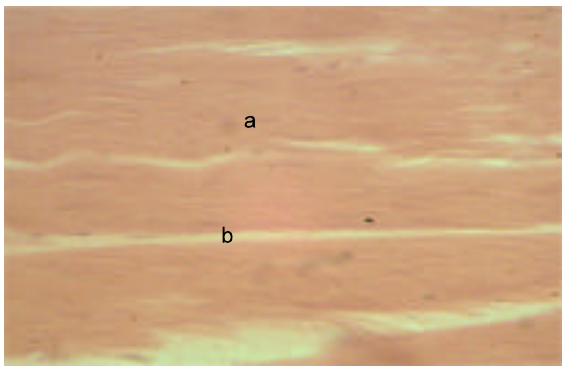

Fig. 3: Histological section in pectoral muscle showing: (a) Longitudinal striated muscle fibers, (b) Endomysium, (H&E) stain 100x

studies carry out by Jeyaprakash and Chinnaswamy (2005) that when $CdCl_2$ given orally to albino rats caused periportal inflammation, fatty change, congestion of

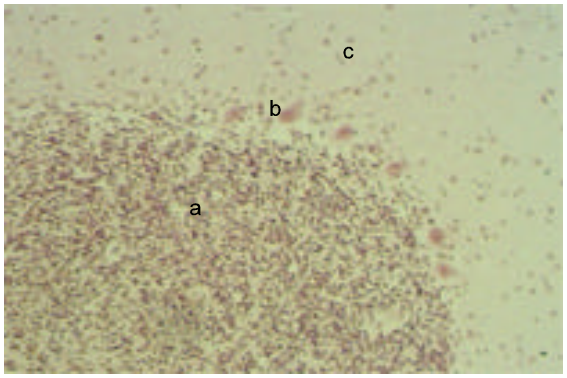

Fig. 4: Histological section in cerebellum showing: (a) Granular layer, (b) Purkinji cell layer, (c) Molecular layer, (H&E) stain 100x

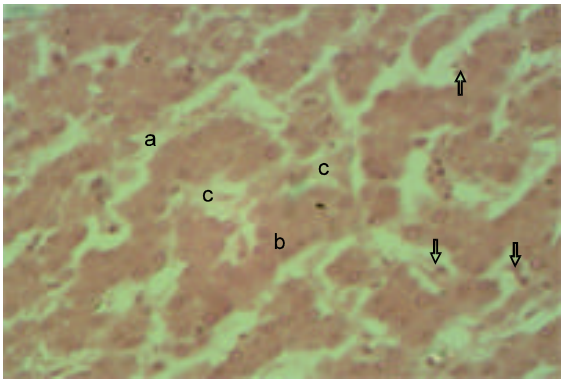

Fig. 5: Histological section of liver showing: (a) Distended sinusoid, (b) Cord of hepatocytes, (c) Amyloid deposit, (d) (Arrows showed coffer cells) (H&E) stain 400x

sinusoids and micro vesicular steatosis in liver, also there were cellular glomeruli, congestion of blood vessels and tubular necrosis in kidneys. Hidetoyo *et al*. (2002) and Jin *et al*. (1987) find out that oral administration of $CdCl_2$ at 50 ppm for 7 days caused renal damage by impairment tubular cells by Cd. Because once Cd is absorbed into the liver from the digestive tract, it stimulate the synthesis of MT (Metallothionein) in the organs and produces MT bound Cd (MT-Cd) that transfer to the kidneys via the blood stream and filtered through the glomerular membrane to reabsorbed in the proximal tubular cells. MT-Cd shows strong nepherotoxicity after pinocytosis and accumulation in the proximal tubular cells (Nordberg, 1984). Naovarat *et al*. (2011) mentioned that MT-Cd formed from necrotic hepatocytes in response to Cd exposure. While T_5 (infected uninjected group) the specimens of liver and kidney showed pathological changes involved amyloid deposition between distended sinusoid, congestion of central vein with RBC

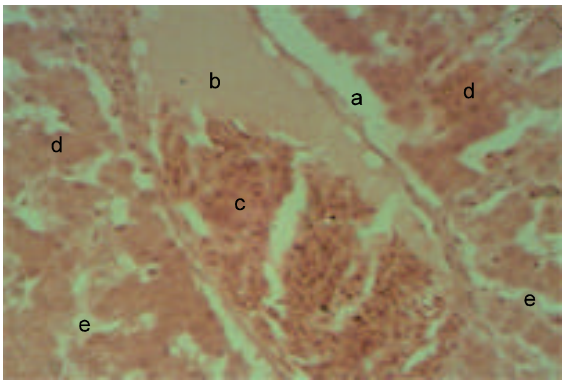

Fig. 6: Histological section of liver showing: (a) Central vein, (b) Amyloid deposit, (c) EBC, (d) Hepatic cord, (e) sinusoid, (H&E) stain 400x

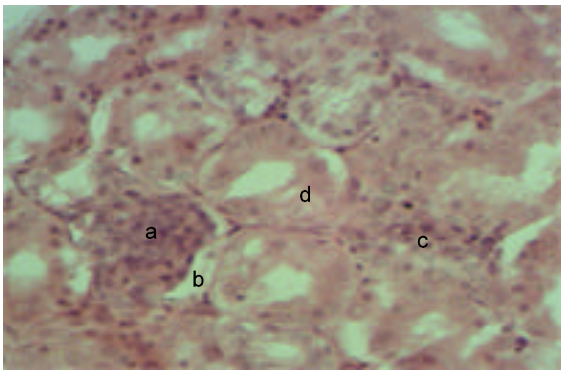

Fig. 7: Histological section of kidney (cortex) showing: (a) Glomerulus tuft, (b) Bowman space, (c) Congested interstitial tissue, (d) Proximal convoluted tubule, (H&E) stain 400x

Table 2: 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

and amyloid deposit, with increased in coffer cells (5) and (6). The kidney showed congestion in cortical interstitial connective tissue and distended of bowman's space of glomeruli (Fig. 7).

Experiment 2: The histopathological results in orally administration treating group showed similar results of injectable administration in experiment 1. Results from Table 2 showed that ashed livers and eggs were empty from any Cd (II) ion residues, which in turn very important for human health. Notice that Cd Maximum Atomic Concentration (MAC) in meat 0.012-0.023, in fish -0.014-0.035, in tomatoes -0.022, in potatoes 0.028 mg\kg (Zimakov, 1980).

Table 3: The effect of giving CAC to layers chickens (*Lohman brown)* on *E. coli* count of small intestine (duodenum)

E. coli count cell\ml	Experimental groups % T_1 (0.0)	T_2 (0.25)	T_3 (0.50)	T_4 (1.0)	T_5 (0.0)	Significance level
Exper.1	8.9 x 10^6a ±0.058	5.1 x 10^6b ±0.058	3.8 x 10^6c ±0.115	2 x 10^6d ±0.115	11.3 x 10^6	*
Exper.2	8.9 x 10^6a ±0.058	6 x 10^6b ±0.058	4 x 10^6c ±0.17	3 x 10^6c ±0.12	11.3 x 10^6	*

Small litters' means: presence of significant differences between groups. *Presence of significant differences at ($p<0.05$) probability level

Statistical analysis of *E. coli* count from small intestine (duodenum) in layer chickens (*Lohman brown)* in Table 3, 1st experiment revealed decreasing of *E. coli* counts T_4, T_3 and T_2 respectively as compared with T_1 and T_5 which had the highest count of *E. coli*. While in 2nd experiment showed decreasing of *E. coli* counts T_4, T_3 and T_2 respectively as compared with T_1 and T_5. That related to the fact of being CAC as antibacterial inhibiting the pathological *E. coli* growth and decrease their counts. Cabuk *et al*. (2003) and Muzahim (2009) mentioned that *E. coli* counts from small intestine (duodenum) decreasing when used anise (*Pimpinella anisum*) in layers, as a result for its antibacterial and antifungal activity. These results were agreed with studies carried out by Jahan *et al*. (2006) when using Cd (II) cystine as antibacterial and antifungal agents (sensitive test) against numbers of bacteria and fungi, for example (*Salmonella typhi*, 36 mm at 80 µg concentration and 24 mm at 50 µg concentration; *Staphylococcus aureus*, 35 mm at 80 µg concentration and 22 mm at 50 µg concentration; *Aspergillus niger*, 39 mm at 600 µg concentration and 33 mm at 300 µg concentration; *Aspergillus flavus*, 34 mm at 600 µg concentration and 28 mm at 300 µg concentration).

CAC 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 1.0% and 0.50% of CAC concentration respectively were effective to protect and treat layers from induced *E. coli* infection safely, as a synthetic chemical prophylactic and antibiotic in layers.

Abbreviations:

1. CAC: [Dinitrato- bis {p-methyl anilino phenyl aceto nitrite} cadmium (II)].
2. (HL): {p-methyl anilino phenyl aceto nitrite}.
3. Cd: Cadmium.
4. *E. coli*: *Escherichia coli*.
5. T_1, T_2, T_3, T_4 and T_5 are representing experimental chicken.

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