A Comparative Histological Study Between Injection and Oral Administration of New Cadmium Aminonitrile 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 nitro-bis ((p-methyl anilino) phenyl acetonitrile] Cadmium (II)]. (2)Hydrate, [Cd(HL)(NO3)2].2H2O 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 (T1, T2, T3, T4 and T5), 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, (T1: 0.25%, T2: 0.50% and T3: 1.0%). After five days E. coli was orally administrated by drinking water for the treatments (T3, T4 and T5) at titer of 8500 x 10^6 cells/ml (highly pathogenic) as causative agent of Salpingitis and Ovaritis. Uninjected uninjected T1 was used as control. In experiment 2, E. coli was orally administrated to T3, T4 and T5 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, (T1: 0.25%, T2: 0.50% and T3: 1.0%) by mixing it with the diet. Uninfected untreated T1 0.0% was used as a control. T5 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 (T1). The specimens of liver and kidney of T5 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). Jayaprakash 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). Yoshihde et al. (2005) mentioned that thyroid gland function of rats affected when treated with CdCl2. 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 heuristically 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 cytoxic effect between administrated orally or as oily injectable preparation.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>30.80</td>
</tr>
<tr>
<td>Wheat</td>
<td>37.00</td>
</tr>
<tr>
<td>Soya bean (40%)</td>
<td>18.00</td>
</tr>
<tr>
<td>Concentrated animal protein (40%)</td>
<td>5.00</td>
</tr>
<tr>
<td>Vitamins and minerals</td>
<td>0.10</td>
</tr>
<tr>
<td>Soya bean oil</td>
<td>0.30</td>
</tr>
<tr>
<td>DCP stone</td>
<td>7.50</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.03</td>
</tr>
<tr>
<td>DCP (18%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mathematical chemical analysis of diet ingredients according to (NRC, 1994) was (metabolized energy: 2752 Kcal/or; mathematical crude protein: 17.50%; lysine: 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 nitrito-bis ((p-methyl anilino) phenyl acetanilide) Cadmium (II)](2)Hydrate, with a chemical formula [Cd (HL):(NO3):2H2O], this compound was synthesized from alpha-aminoanilide 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 48 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 (T1, T2, T3, T4 and T5), each main group divided into 3 subgroups, T5, T3, T4 and T5 were injected subcutaneously in the neck by CAC dose of 0.25%, 0.5% and 1.0% respectively, uninjected T1 was used as control. T5 was important only to identify E. coli infection in infected un.injected chickens. The (Escherichia coli) as Salpingitis and Ovaritis agent has been given to the layers T3, T4 and T5 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 (T1, T2, T3, T4 and T5), each group divided into 3 subgroups. After a week of chicken accumulation the (Escherichia coli) as Salpingitis and Ovaritis agent has been given to the layers T2, T3, T4 and T5 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 T2, T3 and T4 respectively. Untreated, uninfected T1 was used as control.

In both experiments T1 was important only to identify E. coli infection and its counts in direct smears from intestinal tract in infected un.injected 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. Until 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 (Indole 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, sectoned at (5 μm thickness) and stained with hematoxyline- eosin stain (Luna, 1988). To determine the Cd residues in chicken bodies (Exp. 1 and 2), the samples of liver and eggs were collected after 10, 30, 65 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. (2008) 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 T2 occasionally were showing Pathological symptoms that revealed perihepatitis, pericarditis, ascitis, gaseous ovary, atrophied oviduct which are the main pathological symptoms of ovaritis and Salpingitis (Azusa et al., 2007). E. coli cultured on nutrient agar by using samples collected from intestine of T5, 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 coloring below in the test tube and Methylened Red text 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 T1, T2 and T3 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 studies carry out by Jayaprakash and Chinnaswamy (2005) that when CdCl2 given orally to albino rats caused periportal inflammation, fatty change, congestion of
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 CdCl₂ 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 nephrotoxicity 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 T₅ (infected uninjected group) the specimens of liver and kidney showed pathological changes involved amyloid deposition between distended sinusoid, congestion of central vein with RBC 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>
<thead>
<tr>
<th>Samples</th>
<th>Period of collection after injection/day</th>
<th>Samples numbers</th>
<th>Cadmium µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>10</td>
<td>18</td>
<td>-0.027</td>
</tr>
<tr>
<td>Egg</td>
<td>30</td>
<td>18</td>
<td>0.006</td>
</tr>
<tr>
<td>Egg</td>
<td>85</td>
<td>18</td>
<td>-0.027</td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>16</td>
<td>-0.027</td>
</tr>
<tr>
<td>Liver</td>
<td>85</td>
<td>18</td>
<td>-0.029</td>
</tr>
</tbody>
</table>
Table 3. The effect of giving CAC to layers chickens (Loxman brown) on E. coli count of small intestine (duodenum)

| E. coli count
| Experimental groups % |
|----------------------|----------------------|
| cell/ml              | T1 (0.0) | T2 (0.25) | T3 (0.50) | T4 (1.0) | T5 (0.0) |
| Exper.1              | 9.8 x 10^5a | 5.1 x 10^6b | 3.8 x 10^6c | 2 x 10^6d | 11.3 x 10^7 |
|                      | ±0.058   | ±0.068   | ±0.115    | ±0.115   | ±0.12    |
| Exper.2              | 9.8 x 10^5a | 6 x 10^6b   | 4 x 10^6c  | 3 x 10^6c  | 11.3 x 10^7 |
|                      | ±0.058   | ±0.068   | ±0.17     | ±0.12    |

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 (Loxman brown) in Table 3, 1st experiment revealed decreasing of E. coli counts T1, T2 and T3 respectively as compared with T1 and T3 which had the highest count of E. coli. While in 2nd experiment showed decreasing of E. coli counts T4, T5 respectively as compared with T5 and T5. 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 Muzahir (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. (2008) 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 1.355 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).
4. E. coli: Escherichia coli.
5. T1, T2, T3, T4 and T5 are representing experimental chicken.

**REFERENCES**


