Effect Of Apramycin On Pathological, Hematological And Biochemical Changes In Turkey Infected With Coli-Bacillosis

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ABSTRACT

Eighty turkey poults 20 days old were equally divided into 4 equal groups to evaluate the efficacy of Apramycin against the colibacillosis; group1 was kept as control, group 2 received 25mg/kg,b.wt. of Apramycin in drinking water for 5 successive days, the group3 was infected with 3x10⁷ organisms of E.coli (O78) and group 4 was infected with E.coli and treated with Apramycin. Five poults from each group was sacrificed, 1, 15 and 20 day from the beginning of the experiment.

Two blood samples were taken and the serum was separated for hematological and biochemical studies. Tissue specimens were collected for drag- residues assessment and for pathological examination. The poults of group3 shows significant decrease in RBCs, Hb, PCV%, total proteins, albumin, globulins with insignificant increase in A/G ratio, and significant elevation in WBCs, AST, ALT, ALP, Uric acid and Creatinine. These changes were lowered in group 4. The highest Apramycin residues were present in the kidneys, liver, skin and thigh muscles, respectively, then disappeared completely from the examined organ samples after 20 days post treatment. The pathological examinations were coincided with the biochemical examination these changes included degenerative changes in hepatocytes, epithelial lining of renal tubules in addition to coagulative necrosis in both hepatic and renal parenchyma. Congestion and leukocytic infiltrations were noticed. Finally, it could be concluded that the Apramycin is effective against the Colibacillosis, in turkey poults.

INTRODUCTION

Escherichia coli, usually abbreviated to E.coli, is one of the main species of bacteria normally inhabitants of the lower intestines of warm-blooded animals (birds and mammals) (1). Colibacillosis affects poultry industry causing serious economic losses achieved by high mortality and loss of body weight (2). Colibacillosis was associated with various disease conditions (3). The acute form in poultry leads to septicemia and death (4).

Escherichia coli serotype O78 is highly pathogenic for chickens and can induce mortalities within short time (5).

Escherichia coli infection in poultry is associated with pericarditis, perirehepatitis, nephritis, air-sacculitis, peritonitis, panophthalmitis and omphalitis. There were also degenerative changes in most organs of the affected chickens, represented by vacuolar and hydropic degenerations in addition to necrosis of hepatocytes and renal parenchyma. Blood vessels of most organs were congested, sometimes hyperplasia of their walls. Colibacillosis causes an elevation of AST, ALT and ALP, in addition to increase in Total proteins, Albumin, Globulins, Uric acid and Creatinine. (6).

Aminoglycoside antibiotics play an important role in treatment E.coli in poultry (7). Apramycin is a broad-spectrum aminoglycoside antibiotic produced by a strain Streptomyces tenebrarius strain (8). It is extracted from the fermentation medium as Apramycin sulphate. A microbiological assay is used to determine its
activity as equivalents of Apramycin base. Apramycin was used in the treatment of Colibacillosis in poultry. Apramycin leads to a decrease in RBCs count, Hb, PCV. An increase in WBCs count due to E.coli infection, decreased after Apramycin administration to infected chickens (9,10).

The present work was conducted to throw light on the efficacy of Apramycin in controlling E.coli infection in turkey poult's and their effects on hemato-biochemical parameters and pathological changes in some internal organs associated with this infection and treatment, as well as detection of Apramycin residues in internal organs.

MATERIAL AND METHODS

Drugs

Apramycin: Apramycin sulfate soluble powder obtained from Unipharma Company for medical industry. Its traditional name is Apracin, each 150 gm of this powder contains 78 gm Apramycin sulphate. Apramycin is an aminoglycoside antibiotic produced by Streptomyces tenebriosis.

Experimental Turkey Poults

A total of 80 healthy one day old turkey poults were obtained from local commercial hatchery. Turkey poult's were floor reared under hygienic condition, fed on balanced ration free from any medications and given water ad libitum. All turkey poult's were subjected to bacteriological examination and proved to be free from infection before E.coli inoculation.

Microorganisms

E.coli strain (O.78) used in this study was isolated from a field case from broiler chickens farms infected with colisepticemia in Sharkia Governorate. Identified and serotyped as O78 (11,12).

E. coli inoculum: Broth culture was standardized to give bacterial suspension containing 3X 10⁷ viable organism/ml of E.coli O78 using MacFerland tube. Each bird was given 0.3 ml via nasal route (13).

Experimental Design

At 20th day of age turkey poult's were divided into four equal groups (20 each). 1st group turkey poult's were left non infected non treated (-ve control), 2nd group healthy turkey poult's treated with Apramycin (25 mg/kg B.wt in drinking water for 5 successive days), 3rd and 4th groups were experimentally infected with E.coli at the 20th day of age, 3rd group infected, non-treated turkey poult's (+ve control), 4th group infected turkey poult's and treated with Apramycin at the same dose and period. Treatment started 24 hrs post infection. All diseased turkey Poult's were left under observations during the experimental period to record the clinical symptoms and mortality rate.

Sampling

Blood Samples

Five birds from each group were slaughtered at 1st, 15th and 20th day post treatment for collection of 2 blood samples. The 1st sample was taken in a tube containing EDTA and used for hematological studies, the 2nd sample was taken to obtain clear serum for estimation of AST, ALT and ALP, T. proteins, serum albumin, globulins mathematically, uric acid and creatinine according to (14).

Media Used: MacConkey's agar, nutrient agar, MacConkey's broth and Nutrient broth

Swabs for re-isolation of E.coli (O78)

Sterilized swabs from trachea, lung, heart, liver and air sacs were taken from sacrificed turkey poult's for bacteriological examination for re-isolation of inoculated E.coli. Swabs were incubated in nutrient broth at 37°C for 24hrs, then subcultured into MacConkey's agar then incubated at 37°C for 24hrs (15). Re-isolated bacteria were identified biochemically and serology (16).
Tissue Samples for drug residues

Samples were taken from slaughtering turkeys post treatment from thigh muscles, liver, kidney and skin for determination of Apramycin residues (17).

Preparation of medium and test plates

Per 100 ml of Apramycin medium at 48°C, 1 ml of micrococcus spore suspension (10^7 spore/ml) was added to obtain a density of 10^4 spore/ml. The medium was shaked well and 13 ml^3 of the prepared medium was poured into a number of petri dishes (1 cm depth). The plates were left at room temperature till complete solidification, then 6 pores were made on each plate using sterile borer with outside diameter 8 mm.

Procedure

Two plates spilled with different concentrations of Apramycin were used, and then plates were incubated at 30°C for 24 hrs. The width of inhibition zones were recorded then marking a curve between concentration of Apramycin and width of inhibition zone. The same procedure was done for turkey Pouls tissues and the concentration of Apramycin was determined by comparing with those obtained by calibration curve.

Specimens for histopathological studies

Specimens from the liver and kidneys were taken from sacrificed birds then fixed in 10% neutral formalin solution and embedded in paraffin. Five microns thick sections were prepared and stained by H&E then examined microscopically (18).

5) Statistical analysis: The obtained data was analyzed (19).

RESULTS

The obtained results were tabulated in tables 1, 2, 3, 4 and 5 and Figures 1 to 6.

Table 1. Effect of Apramycin on the mortality rate, pathological lesions and reisolation in healthy and E.coli infected turkey pouls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total No</th>
<th>Mortality</th>
<th>Lesion scores %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Non-inf., non treated</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non Inf. Treated</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inf. Non treated</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Inf. Treated</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Re-isolation of E.coli from infected non treated and infected treated turkey pouls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of Examined pouls</th>
<th>Re-isolation of E. coli for internal organs</th>
<th>Trachea</th>
<th>lung</th>
<th>Heart</th>
<th>Liver</th>
<th>Air sacs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Non-inf., non treated</td>
<td>20</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>Non</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Inf., Treated</td>
<td>20</td>
<td></td>
<td>20</td>
<td>100</td>
<td>16</td>
<td>80</td>
<td>12</td>
</tr>
<tr>
<td>Inf., Non treated</td>
<td>20</td>
<td></td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3. Effect of Apramycin on Blood picture of healthy and infected turkey poults with E.coli

<table>
<thead>
<tr>
<th>Group</th>
<th>Healthy turkey (n=5)</th>
<th>Healthy treated turkey (n = 5)</th>
<th>Non treated</th>
<th>Diseased turkey (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post treatment (days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>RBCs (106/µl)</td>
<td>5.73±0.37</td>
<td>4.06±0.48*</td>
<td>3.82±0.32**</td>
<td>4.12±0.42*</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>10.70±0.58</td>
<td>8.33±0.63*</td>
<td>7.94±0.77*</td>
<td>8.31±0.62*</td>
</tr>
<tr>
<td>PCV%</td>
<td>28.42±0.94</td>
<td>25.14±0.92*</td>
<td>24.55±0.99*</td>
<td>26.03±0.24*</td>
</tr>
<tr>
<td>WBCs(106/µl)</td>
<td>15.38±0.91</td>
<td>18.96±0.97*</td>
<td>19.21±0.99*</td>
<td>1796±0.62*</td>
</tr>
</tbody>
</table>

*Significant at < 0.05

Table 4. Effect of Apramycin on liver and kidney functions in healthy and infected turkey poults with E.coli (n = 5)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Liver enzymes(u/ml)</th>
<th>Liver Functions</th>
<th>Protein profile (gm/dl)</th>
<th>Kidney Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST</td>
<td>ALT</td>
<td>ALP</td>
<td>T. proteins</td>
</tr>
<tr>
<td>Healthy Poults</td>
<td>Non treated</td>
<td>77.12</td>
<td>30.21</td>
<td>110.04</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>±1.05</td>
<td>±0.58</td>
<td>±0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.90*</td>
<td>±0.72**</td>
<td>±1.64*</td>
</tr>
<tr>
<td>Diseased Poults</td>
<td>Non treated</td>
<td>81.15</td>
<td>33.11</td>
<td>115.06</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>±1.13*</td>
<td>±0.83*</td>
<td>±1.92*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.70*</td>
<td>±0.52*</td>
<td>±1.30*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±4.85</td>
<td>±0.39*</td>
<td>±1.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.58</td>
<td>±0.28</td>
<td>±1.28</td>
</tr>
</tbody>
</table>

*Significant at < 0.05

Table 5. Apramycin residues (µg/g) in fresh turkey poults tissues and organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Healthy poults treated with Apramycin</th>
<th>Diseased Poults treated with Apramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days post slaughter (days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.22±0.05</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>1.07±0.11</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.07±0.16</td>
<td>0.50±0.07</td>
</tr>
<tr>
<td>Skin</td>
<td>0.31±0.05</td>
<td>0.10±0.02</td>
</tr>
</tbody>
</table>
Pathological finding

**Groups 1,2:** Control and received Apramycin. Neither Gross nor microscopical abnormalities were seen.

**Group 3,4:** Infected (non-treated) and infected (treated).

**Macroscopically**

The liver and kidneys of Group (3) showed severe congestion, firm and focal whitish necrotic foci, moreover, thickening of the hepatic and kidney capsules were noticed, in few cases, focal hemorrhagic spots were seen in the renal cortex. The liver and kidneys in most cases of group 4 were dark red to brownish in color and enlarged in size.

**Microscopically**

The liver of group 3: showed congestion of the hepatic blood vessels, the liver revealed thrombosis and thickening of the wall of the portal vein, besides leukocytic aggregations in hepatic parenchyma and hemorrhage. (Fig.1). Degenerative changes coagulative necrosis of hepatic parenchyma which represented by pyknosis karyorrhexis and karyolysis of the nuclei of hepatocytes. (Fig.2).

The kidneys of group 3: showed hypercellularity of the glomeruli in addition to degenerative changes which represented by vacuolar and hydropic degenerations and perivascular edema (Fig.3). In some cases, focal coagulative necrosis of the epithelial lining renal tubules, hyperplasia and thickening of the renal capsule was also observed (Fig.4). Congestion of the renal blood vessels and leukocytic aggregations in renal parenchyma was also observed. Most infected cases with E.coli, the lining renal tubules showed cloudy swelling and individual cell necrosis which represented by pyknosis and karyorrhexis of their nuclei (Fig.5).

The lesions of group 4 were alleviated than those described in group3. Mild degenerative changes (vacuolar and hydropic types), slight congestion and few round cells infiltrations were visualized. (Fig.6).
Fig. 1. Electronmicrograph of the liver of Group 3 showing congestion of the hepatic blood vessels and thrombosis and thickening of the wall of the portal vein in addition to leukocytic aggregations in hepatic parenchyma H&E × 200.

Fig. 2. Electronmicrograph of the liver of Group 3 showing focal coagulative necrosis of hepatic parenchyma H&E × 100.
Fig. 3. Electromicrograph of the kidney of Group 3 showing vacuolar and hydropic degenerations of renal tubules in addition to hypercellularity of glomeruli and perivascular edema H&E × 200

Fig. 4. Electromicrograph of the kidney from infected turkey poults treated with Apramycin showing Focal coagulative necrosis of the epithelial lining renal tubules with hyperplasia of the renal capsule H&E × 200
Fig. 5. Electronmicrograph of the kidney: y of Group (3) showing cloudy swelling and individual cell necrosis of the epithelial lining of renal tubules which represented by karyorrhexis and karyolysis of their nuclei H&E × 300

Fig. 6. Electronmicrograph of the liver of Group 4 showing congestion of the hepatic blood vessels and sinusoids H&E × 200
DISCUSSION

Turkey poult experimentally infected with E. coli displayed clinical signs as loss of appetite, weakness, depression, sneezing, cough, depression, watery diarrhea and 25% mortality. The same signs were recorded in broiler chickens (12, 20). Mortality rate in E.coli infected broiler ranged from 10-40% (21, 22) who found that mortality rate in broilers infected with E.coli was 40%.

Effects of Apramycin on blood picture in healthy and infected turkey poult with E.coli showed significant reduction in RBCs, Hb, PCV and leukocytosis. (Table 3). Same changes were recorded which showed that apramycin induced significant decrease in RBCs, Hb, PCV in broilers. (25). Aminosidine another aminoglycoside was found to induce decrease in RBCs, Hb and PCV and leukocytosis. (24). Our results proved previous observations which recorded that broilers infected with E.coli showed significant decrease in RBCs, Hb and PCV. (25). Reduction in erythrogram may be due to lipopolysaccharide toxin secreted by E.coli which inhibits bone marrow cells and nephrotoxicity decrease erythropoetin blood level which followed by decrease RBCs formation (26). Also, it has been shown that bacterial endotoxins cause intra-vascular destruction of erythrocytic cells leading to haemolysis with break down of Hb (27). Leukocytosis in E. coli infected birds may be due to inflammatory response in the gastrointestinal tract due to bacterial infection (28).

Analysis of protein profile of the healthy turkey poult treated with Apramycin and those infected with E. coli showed significant decrease in total proteins, albumin and globulins (Table 4). Similar findings were previously cited by (29) which showed that apramycin evoked a significant decrease in proteins, albumin and globulins in rabbits. Decrease in serum protein picture in turkey poult treated with Apramycin might be due to damage in hepatic cells (30). Broiler chickens infected with E.coli showed significant decrease in serum total proteins, albumin and globulins (2, 31, 32). Decrease in albumin in birds infected with E. coli could be due to liver damage in which liver is the sole site of albumin synthesis (36).

In the present study, healthy turkey poult treated with Apramycin and infected with E.coli showed significant increase in serum AST, ALT and ALP, uric acid and creatinine (Table 4). Our results were supported by previous study (23) which indicated that healthy rabbits treated with Apramycin showed significant increase in AST, ALT, ALP, uric acid and creatinine. Increase in uric acid and creatinine post administration of Apramycin may be due to accumulation and retention of Apramycin in proximal tubular cells inducing kidney damage (33). Our results are in accordance with the results obtained by previous authors (34, 35), where they found that E.coli infection in broiler induce a significant increase in AST, ALT, uric acid and creatinine, in broilers infected with E.coli which may be due to liver damage induced by the organism and its toxins which lead to escape of these enzymes into serum (36).

Our findings revealed that, Apramycin residues in the examined samples of turkey poult's liver, muscle, kidney and skin were very high at 1st day of clearance period, very low at 15th days post treatment and completely disappeared from all examined organ at 20th day post treatment. The highest levels of Apramycin residues were recorded in the kidney followed by liver then skin and muscle (Table 5). The obtained results nearly coincide with those previously reported (37) which showed that, the average total residues of Apramycin at 1st day of withdrawal were 3.23, 0.42, 0.20 and 0.07 mg/kgm in kidney, liver, skin and muscle, respectively. After 14 days, average of total residue had declined to 0.47, 0.08, 0.03 and 0.02 mg/kg in the same respective tissues. The highest Apramycin residue was present in the kidneys followed by liver (8).

Gross pathological lesions present in our study in group 3 (infected group) were pericarditis, pericarditis, air sacculitis misshaped and congestion these lesion scores percentages were decreased in group 4 (infected
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and treated. Poultry infected with E.coli manifested the previously mentioned lesions (25). Healthy turkey poult treated with Apramycin showed pathological lesions as congestion of hepatic blood vessels with vacuolar degeneration of hepatocytes; kidney showed focal coagulative necrosis of renal tubular epithelium. The histopathological findings could be due to septicemic effect of E. coli upon the blood vessels, serous membranes and the parenchymatous organs (38). Same lesions were recorded previously (39) in Guinea pigs given Apramycin. Liver showed congestion of hepatic sinusoids, vacuolation in some hepatic lobules and hydropic degeneration, coagulative necrosis and lymphocytic infiltrations. Kidney showed cystic dilatation, hydropic degeneration of some renal tubules and desquamation of some epithelial lining of the renal tubules in turkey pouls infected with E.coli. Our results were nearly similar to that recorded by several authors (40 - 42).

CONCLUSION

From this study we concluded that, Apramycin is effective for treatment and improvement of the liver and kidney functions after infection by Escherichia coli (E.coli 078) in turkey poult. As E. coli infection has adverse effect on liver and kidney functions in addition to degenerative changes and necrosis of hepatocytes and epithelial lining renal tubules. Apramycin residues was detected in kidney, liver and skin respectively and removed completely from body organs after 20 days of Apramycin treatment. It is advised to use Apramycin for E.coli treatment of turkey poult.

coli isolated from fertile and infertile eggs dead-in-shell embryos and chickens with yolk sac infection. Avian Dis. 48(4):81-89.


REFERENCES

chickens inoculated with infectious bronchitis virus and/or E. coli. Avian dis. 36:881-890.


حدث نقص معيون في عدد كريات الدم الحمراء وتركز الهيموگلوبين، وحجم خلايا الدم المرصوسة البروتين الكلى. الزلال-الجلوبيولين مع حدوث زيادة معيونية في عدد كريات الدم البيضاء إضافة لانزيم الأسرتات الأمينوتراستفيزيز-الألاتين الأمينوتراستفيزيز والفسفاتيز القاعدي. حمض البوليك والكرياتينين.

وقد دلت النتائج الدراسة على أن الإبراميسين له أبايا في نسبته عضلات الفخذ. الكبد الكلى والجلد وكانت بنسبة عالية بعد اليوم الأول وبنسبة مخفضة عند اليوم الخامس عشر بعد نهاية العلاج بينما اختفت أبايا الإبراميسين عند اليوم 20 بعد نهاية العلاج. وكان أعلى مستوى للبدلا في الكلي بليه الكبد ثم الجلد واخيرا عضلات الفخذ. والفحص الظاهرى للكبد والكليه وجد تضخم واحتكان سواء في كتاكين كلس الميليم والمعالجة بالإبراميسين أو المصاب به بال mikroob القولونى العصوى. أما الفحص المجهرى للاعصابات الداخلة لكتاكين الكلس والمعالج بالإبراميسين أو العصوبة بال mikroob القولونى العصوى وجدت آفات بالكوليكية عباره عن احتقان بالبسمات الدموية مع وجود نخ خثرى بالخلايا الكلبية. أما الكلى فوجد نكس مع دوز نخر خثرى بالبسمات الكلوية. وقد اظهرت المجموعة المصابه بال mikroob القولونى العصوى ومعالجة بالإبراميسين تغيرات بالكوليكية طفيفه وانخفاض معدل النفوق إلى 5% وعودة وظائف الكبد والكلي إلى المستوى الطبيعي.

نستخلص من تلك الدراسة أن الإصابة بال mikroob القولونى العصوى في بدارى ضمن الرومي احدثت بعض التأثيرات العكسية على صورة الدم ووظائف الكبد والكلي، وادي استخدام الإبراميسين إلى تحسين الحالة الصحية لكتاكين الرومي بعد العلاج بـ 15 يوم وينصح باستخدام الإبراميسين الجرعة العلاجية لعلاج الإصابة بال mikroob القولونى العصوى كما ينصح بعدم دخول الدواجع المعالجة إلا بعد مرور 30 يوم من نهاية العلاج.