Some Biochemical and Pathological Changes in Turkey Induced
By Gentamicin and Their Modulation with Vitamin E

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ABSTRACT

The present study was designed to investigate the protective role of vitamin E against gentamicin toxicity. A total of 80, 4 weeks-old turkey poult were divided into 4 groups. 1st group healthy non-treated turkeys (control group), 2nd group gentamicin (5mg/kgm B.wt/day) treated, 3rd group vitamin E (10mg/kg B.wt/day) treated, 4th group treated with gentamicin plus vitamin E. Five turkeys poult each group were sacrificed at 1st day post treatment, blood samples and Specimem from internal organ were taken for hematobiochemical and histopathological study. Five turkey poult from each group were weighted at start and 1st day post treatment for detection effect of the durgs in body weight.

The present work revealed that, gentamicin induced a significant reduction in RBCs count, Hb, PCV %, total protein, albumin, globulin and rise in AST, ALT, ALP, uric acid, and creatinine beside insignificant rise in WBCs count and body weight in turkey at 1st day post treatment. Vitamin E induce significant rise in RBCs count, Hb, PCV % total WBCs count, total protein, albumin globulin and insignificant increase in body weight, AST, ALT, ALP, uric acid, creatinine and body weight in turkey at 1st day post treatment.

Necropsy revealed swollen kidneys and liver with hemorrhagic streaks on its surface. Petechial hemorrhages present on skeletal muscles. Microscopically, kidneys showed degenerative changes and necrosis of the renal tubules. Liver showed severe vacuolar and hydropic degeneration of the hepatic parenchyma.

Oral administration of vitamin E to turkey poult gentamicin treated caused improvement in hematobiochemical parameters and pathological lesions.

From the results, it can be concluded that gentamicin is a highly toxic to turkey birds but vitamin E has potential protective effects against gentamicin toxicity.

INTRODUCTION

Aminoglycoside is a group of bactericidal antibiotics. Gentamicin is an amino-glycoside antibiotic isolated from micromonospora purpura (1). It is widely used for treatment of infections with G-ve bacteria (2). Gentamicinwe an effective drug used to control many diseases in poultry, however, its clinical use is partially limited due to its toxicity (3). The mechanism of action of gentamicin involves irreversible inhibition of bacterial ribosomes and impairs protein synthesis, (4). Gentamicin toxicity results from accumulation and retention of aminoglycoside in proximal convoluted tubular cells (5).

Vitamin E includes 8 isomers in 2 classes designated (tocopherol & tocotrienols) (6). Vitamin E has antioxidants effect in the body (7). Antioxidant activity of vitamin E contributes to stabilization of the oxidation sensitive fatty acids in the cellular metabolism. Free radicals catalyze lipid peroxidation is a continuous process, causing damage to cellular and intracellular structures, vitamin E inhibit these processes (8). Vitamin E stimulates immune system of animals (9).
The objective of this study was to illustrate the harmful effect of gentamicin and to evaluate the possible protective effect of vitamin E on hematological and some serum biochemical parameters in turkey poult.

MATERIAL AND METHODS

Drugs

1- Gentamicin (Garamycin®) Memphis Co. intramuscular injection. Each ml of solution contains 40-mg Gentamicin sulfate.

2- Vitamin E was obtained as a viscous oil of vitamin E (Alpha-Tocopherol): It is available as soft gelatin capsules (400 I.U.).

Birds and Experimental design

A total of 80, 4 weeks old mixed breed turkey poult were used in this investigation. Turkeys were floor reared in separate units along, fed on a balanced ration free from any medication and water was provided ad-libitum. Turkeys were divided into four equal groups (20 birds each). 1st group was healthy turkeys non-treated (control group). 2nd group healthy turkeys injected IM with gentamicin (5 I.U /kg b.wt/day) for 5 successive days (10), 3rd group healthy turkeys orally treated with vitamin E (10 I.U /kg b.wt /day) for 5 successive days and 4th group healthy turkeys treated with gentamicin and vitamin E for the same previous dose and period.

Blood samples

At 1st day post treatment 5 birds from each group were slaughtered and 2 blood samples were taken from each bird, 1st sample was taken in tube contain EDTA for hematological study (11), 2nd sample was taken for obtaining serum for detection of total protein (12), albumin (13), Globulin was calculated as difference between total protein and albumin, AST and ALT (14), ALP (15), uric acid (16) and creatinine (17) were determined.

Body weight

Turkeys in all groups were weighted individually at the start of the experiment and at 1st day post treatment for calculation weight gain and feed conversion rate.

Histopathological studies

Specimens from liver and kidneys were taken from slaughtered bird then fixed in 10% neutral formalin and embedded in paraffin. Sections of 5 microns thickness stained by H&E and microscopically examined (18).

Statistical analysis

Obtained data were statistically analyzed (19).

RESULTS

The present work revealed that, gentamicin induced a significant decrease in erythrocyte count, hemoglobin, packed cell volume %, total protein, albumin, globulin and a significant increase in AST, ALT, ALP, uric acid, and creatinine beside insignificant rise in total leukocytoc count and body weight in turkey at 1st day post treatment. Vitamin E induce significant increase in erythrocyte count, hemoglobin, packed cell volume%, leukocytic count, total protein, albumin, globulin and insignificant increase in body weight, AST, ALT, ALP, uric acid and creatinine in turkey at 1st day post treatment. (Tables, 1 -3)

Turkey poult treated with gentamicin only (2nd group) grossly, showed the liver and kidneys were congested, enlarged, hemorrhagic and the kidneys pale in its colour. The heart of most cases undergoes pericarditis. Microscopically, liver exhibited various degenerative changes with severe congestion of the portal blood vessels and hepatic sinusoids and portal leukocytic infiltration (Fig. 1). The hepatic cells showed clear large necrotic area
beside heterophils and lymphocytes (Fig. 2). Other cases the hepatic cells suffered from telengiectasis (Fig. 3). The renal tubules showed intense necrotic changes in the tubular epithelium with congested capillaries (Fig. 4), other cases undergo hemorrhages, necrosis and leukocytic infiltration were seen (Fig. 5). Vacuolar and hydropic degeneration were seen in some cases (Fig. 6). The heart exhibited severe pericarditis that represented by heterophilic infiltration, congestion of some blood vessels and edema (Fig. 7). Other cases of the cardiac tissue suffered from edema and lymphocytic infiltration (Fig. 8). Turkey poult treated with gentamicin and vitamin E (4th group) microscopically, the hepatic cells exhibited mild coagulative necrosis (Fig 9) and apparently normal hepatic parenchyma (Fig 10) Kidneys of turkey poult treated with gentamicin and vitamin E (4th group) with mild degenerative changes (Fig 11). Heart of turkey poult treated with gentamicin and vitamin E (4th group) exhibited mild pericarditis (Fig 12) and apparently normal myocarditis (Fig 13).

Table 1. Effect of gentamicin and vitamin E on erythrogram of broiler chickens (n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Erythrogram</th>
<th>WBCs (106/c.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>RBCs (106/c.mm) 10.54±0.07</td>
<td>30.27±0.80</td>
<td>17.48±0.69</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5.37±0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E.</td>
<td>7.02±0.57*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genta &amp; Vit.E.</td>
<td>4.18±0.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at P ≤ 0.05 ** Significant at P ≤ 0.01

Table 2. Effect of gentamicin and vitamin E on protein picture of broiler chickens (n=5):

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Albumin (gm/dl)</th>
<th>Globulin (gm/dl)</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.36±0.42</td>
<td>2.32±0.20</td>
<td>2.04±0.21</td>
<td>1.11±0.10</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.78±0.44*</td>
<td>1.51±0.19</td>
<td>1.27±0.18*</td>
<td>1.14±0.14</td>
</tr>
<tr>
<td>Vitamin E.</td>
<td>6.29±0.31**</td>
<td>3.43±0.35*</td>
<td>2.86±0.26*</td>
<td>1.17±0.19</td>
</tr>
<tr>
<td>Genta &amp; Vit.E.</td>
<td>4.59±0.47</td>
<td>2.54±0.38</td>
<td>2.09±0.30</td>
<td>1.19±0.24</td>
</tr>
</tbody>
</table>

Table 3. Effect of gentamicin and vitamin E on biochemical parameters of broiler chickens (n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Liver enzymes</th>
<th>Kidney function (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>AST (U/L)</td>
<td>ALT (U/L)</td>
<td>ALP (U/L)</td>
</tr>
<tr>
<td></td>
<td>83.17±1.63</td>
<td>25.68±0.82</td>
<td>119.30±1.30</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>89.64±1.41</td>
<td>30.16±0.96*</td>
<td>122.43±1.42*</td>
</tr>
<tr>
<td>Vitamin E.</td>
<td>84.05±1.35</td>
<td>27.02±0.79</td>
<td>120.18±1.80</td>
</tr>
<tr>
<td>Genta &amp; Vit.E.</td>
<td>84.37±1.55</td>
<td>27.62±0.98</td>
<td>120.38±1.95</td>
</tr>
</tbody>
</table>

*Significant at P ≤ 0.05 ** Significant at P ≤ 0.01

Table 4. Effect of gentamicin and vitamin E on body weight pre (n=5).

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Control group</th>
<th>Gentamicin</th>
<th>Vitamin E.</th>
<th>Genta &amp; Vit.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial gm at 28 day of age</td>
<td>869.49±7.39</td>
<td>866.04±3.59</td>
<td>870.16±6.40</td>
<td>865.03±9.60</td>
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<tr>
<td>Final gm at 1st day post treatment</td>
<td>910.46±8.92</td>
<td>912.18±7.50</td>
<td>915.39±5.58</td>
<td>916.17±7.22</td>
</tr>
<tr>
<td>Gain gm</td>
<td>40.97±0.35</td>
<td>46.14±0.49</td>
<td>45.23±0.63</td>
<td>51.14±0.53</td>
</tr>
</tbody>
</table>
Fig. 1. Photomicrograph of the liver of turkey poult treated with gentamicin only (2nd group) showing severe congestion and leukocytic infiltration (H&EX300)

Fig. 2. Photomicrograph of the liver of turkey poult treated with gentamicin only (2nd group) showing large area of coagulative necrotic and leukocytic infiltration (H&EX300)

Fig. 3. Photomicrograph of the liver of turkey poult treated with gentamicin only (2nd group) showing telangiectasia (H&EX300)

Fig. 4. Photomicrograph of the kidneys of turkey poult treated with gentamicin only (2nd group) showing severe necrosis of the tubular epithelium (H&EX300)
Fig. 5. Photomicrograph of the kidneys of turkey poult treated with gentamicin only (2nd group) showing necrosis, hemorrhages and focal leukocytic aggregation among the renal tubules (H&EX 300)

Fig. 6. Photomicrograph of the kidneys of turkey poult treated with gentamicin only (2nd group) showing hydropic degeneration in the epithelium lining the renal tubules (H&EX 300)

Fig. 7. Photomicrograph of the heart of turkey poult treated with gentamicin only (2nd group) showing severe pericarditis (H&EX 300)

Fig. 8. Photomicrograph of the heart of turkey poult treated with gentamicin only (2nd group) showing edema and lymphocytic infiltration (H&EX 1200)
Fig. 9. Photomicrograph of the liver of turkey poult treated with gentamicin and vitamin E (4th group) showing mild coagulative necrosis (H&EX300)

Fig. 10. Photomicrograph of the liver of turkey poult treated with gentamicin and vitamin E (4th group) showing apparently normal hepatic parenchyma (H&EX300)

Fig. 11. Photomicrograph of the kidneys of turkey poult treated with gentamicin and vitamin E (4th group) showing mild degenerative changes (H&EX300)

Fig. 12. Photomicrograph of the heart of turkey poult treated with gentamicin and vitamin E (4th group) showing mild pericarditis (H&EX1200)

Fig. 13. Photomicrograph of the heart of turkey poult treated with gentamicin and vitamin E (4th group) showing apparently normal myocardial muscles (H&EX1200)
DISCUSSION

Gentamicin induced significant decrease in total RBCs count, Hb, PCV, beside in significant increase in WBCs count but vitamin E induce a significant increase in RBCs, Hb, PCV and WBCs. Anemia induced by gentamicin may be due to erythropoietin deficiency following injury to the kidney by gentamicin, site of erythropoietin production (20). Also gentamicin can chelate iron and decreased iron level may interfere with Hb biosynthesis (21). Same changes in blood picture were induced by aminoglycoside antibiotics in chickens (22,23). Another aminoglycoside antibiotic (netilmicin): induced significant decrease in RBCs count, Hb, PCV (24). Vitamin E induced significant increase in RBCs, Hb, PCV in rabbits (25). Increase in hemogram post vitamin E treatment may be due to that vitamin E protects the biological membrane from oxidative damage with consequent beneficial effect in preservation and keeping erythrocytes from any hazard or damage effect with consequent increase in erythrocyte (26).

In the present study, gentamicin induces a significant reduction in total protein, albumin and globulin in turkey. Meanwhile, vitamin E induces significant elevation in serum total protein, albumin and globulin. This results in agreement with the results observed (27-28) in chicken. Another aminoglycoside (netilmicin): induces significant decrease in total protein in rats (29). Reduction in albumin post gentamicin treatment may be due to destructive effect of gentamicin on liver cells producing albumin (31). Similar trend was reported that vitamin E induced significant rise in serum total protein, albumin and globulin in chicken. Serum protein picture post vitamin E treatment may be due to increased absorption of globulin and essential protein digested products and improve protein synthesis in liver (32).

In the present work, gentamicin induced significant increase in AST, ALT, ALP, uric acid and creatinine in turkey poult but vitamin E induced insignificant increase in AST, ALT, ALP, uric acid and creatinine. These results come in harmony with the results turkeys (33) and in broilers (34). Gentamicin is rapidly excreted by glomeral filtration and the small % is reabsorbed by the epithelial cells of the proximal tubuls, lead to accumulation within the renal cortex. Accumulation of the reabsorbed drug leads to nephrotoxicity (35). Therefore the nephrotoxic effect of aminoglycosides due to their marked accumulation and retention in proximal tubular cells (2). Rise of liver enzymes, uric acid and creatinine induced by gentamicin was mainly due to damage in liver and kidney (30). In the same direction the rise in uric acid and creatinine may be due to nephrotoxic effect of gentamicin (36). Rise in liver enzymes, uric acid and creatinine post gentamicin treatment may be due to degenerative changes in liver and tubular necrosis in the kidneys (37). Gentamicin induces desquamation and atrophy of tubular epithelial cells in rats (38). Moreover, (39) stated gentamicin induce severe tubular necrosis in rats associated with rise serum urea, creatinine. Similar structural changes in liver and kidney were observed by (28). Gentamicin induces acute tubular necrosis in kidney and liver fatty change, vacuolar degeneration, necrotic areas and cellular infiltration around portal triads. Vitamin E induce insignificant rise in liver enzymes, creatinine and uric acid in chicken (40). Also, vitamin E induce insignificant rise in liver enzymes (41).

The insignificant increase in the body weight and weight gain in gentamicin and vitamin E, in turkeys run parallel with recorded that antimicrobials produced an increase in the growth rate in growing chicks, increase body weight gain with improved feed conversion through inhibiting pathogenic organisms which damage gut epithelium impairing food absorption, inhibiting pathogenic organisms which compete for growth factors in the gut and inhibiting organisms producing toxic substances affecting growth (42). Also vitamin E induce improving the efficiency of digestion and increase in body weight in rabbits (43).
Gross alterations observed in our study were in close resemblance to those reported in chickens (27,34). Lesions arised post gentamicin treatment may be due to drug accumulation in the proximal convoluted tubules, leading to loss of brush border integrity and hepatic degenerative changes (45). Pathological changes in the liver post gentamicin treatment are parallel to previous observation (46).

The present data revealed that vitamin E ameliorate toxic effect of gentamicin and improved in hematobiocchemical parameters and pathological lesion. Same results were reported in rats (47). In addition, it has been reported that found vitamin E induce improvement in both hepatic and renal lesion which induced by gentamicin in dogs (48). This may due to the ability of vitamin E to improve the endogenous antioxidants (49) or due to ability of vitamin E to scavenge the free radicals (50).

From the results, it can be concluded that gentamicin is a highly toxic to turkey birds but vitamin E has potential protective effects against its toxicity.

REFERENCES


40. Attia A, Ayat M and El-Zaita A (2005): Role of clay or vitamin E in layer hens fed diets contaminated by lead. 2nd paper: Blood components and lead residues in the
tissues and eggs. Allatt. Takar, 54 (2) 179–190


وأظهرت النتائج أن فيتامين هـ أدي إلى حدوث زيادة في عدد كرات الدم الحمراء، تركيز البيوموجلوبين، حجم خلايا الدم المرصوصة، كرات الدم البيضاء البروتين الكلي، الالبوبمين و الحليبوني وزيادة غير معنوية في، الكرياتينين وحمض الوريك، بالفحص الظاهري للكتاتيب الرومي المرضي مع علاج الفيتامينات فقط (المجموعة الثالثة)؛ وجد أن الكبد والكلى بهما احتقان وتضخم وانزهف كما أن القلب به التهابات في غشاءه. وبالفحص المجهرى للكبد (المجموعة الثانية)؛ وجد تنكسات مخلأ ة واحتقان وارتشاح للخلايا الالتهابية بإضافة إلى أساكن بها تنازح. بالنسبة للكلى توجد بها نخر بالتعددات الكلوية بالإضافة إلى احتقان وارتشاح بالخلايا الالتهابية مع تنكسات مختلفة في بعض الحالات الأخرى. أما بالنسبة للقلب في ورم به بالإضافة إلى التهاب غشاءه. بما بالنسبة للمجموعة الرابعة، توجد بها نخر تدثر خفيف باعتساب خفيف للحبوبية للجلد مع خلايا كبدية طبيعية والكلية بها ارتشاح خلالي بسيط للخلايا الالتهابية والقلب يوجد به التهاب خفيف غشاءه التامور وعصابات القلب، وجدت بحاله سليمة. وتلاحظ أن استخدام الفيتامينات فيتامين هـ أدي إلى عودة هذه الوظائف إلى المستوى الطبيعي في مصل كتاتيب الرومي.

هما سبق واستنادا إلى التغيرات في صورة الدم والتغيرات البيوكيميائية في مصل كتاتيب الرومي والتأثيرات البيولوجية التي أحدثها الفيتامينات يمكن القول أن ذلك العقار له تأثيرات إيجابية على الجسم ولكن استخدام فيتامين هـ أدي يمكنه التغلب على الآثار المكسوة للكتاتيبات لذلك ينصح باستخدام فيتامين هـ للتعامل مع تلك الآثار المكسوة أثناء العلاج بالكتاتيبات.