Effects of Ochratoxin A Feeding in White Leghorn Cockerels on Hematological and Serum Biochemical Parameters and its Amelioration with Silymarin and Vitamin E

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ABSTRACT

The objective of the present study was to evaluate the hematobiochemical effects tempted by ochratoxin A (OTA) in White Leghorn (WL) cockerels and to evaluate the effect of silymarin (SL), vitamin E (VE) and their combination against OTA persuaded pathological alterations in cockerels. A total of 240 day-old WL cockerels were divided into 12 groups A-L having 20 birds in each group and group A was control. Two doses of OTA 1000 and 2000 µg/kg of feed were given to cockerels up to 7 weeks. Silymarin was administered at a dose rate of 10000 mg/kg and Vitamin E at a dose rate of 200 mg/kg alone and in combinations with two doses of OTA. In OTA treated groups total erythrocytes counts, leukocytes count, PCV and Hb were decreased as compared to control, SL and VE groups. Albumen and serum total protein in OTA treated groups were significantly lower as compared to control, SL and VE groups. Serum alanine transferase was significantly increased in OTA fed groups in comparison with control, SL and VE groups. Creatinine and urea were increased in OTA treated groups but were almost normal in SL and VE groups. Results showed that OTA had severe effect on liver and kidney but SL and VE treated groups had normal liver and kidneys showing its hepatoprotective effects. However, at higher dose of OTA this ameliorative effect was partially observed. These agents may be recommended as a remedy for ochratoxicosis.

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INTRODUCTION

Ochratoxin A is an important mycotoxin produced by different Aspergillus and Penicillium species. The presence of ochratoxin A (OTA) in animal feeds raises concerns in poultry and livestock industry due to subclinical intoxications and poor growth in animals (Gentles et al., 1999; Zia et al., 2010). OTA present in the poultry feeds in low to moderate levels induces immunosuppression, decreased bodyweight gains and increased susceptibility to infectious diseases (Saleemi et al., 2010; Mukhtar et al., 2010). OTA has gained considerable attention due to its intrinsic toxicity and its frequent occurrence in feed commodities used in livestock feeds. The main target organ of OTA in poultry, as in other species, appears to be the kidney, although liver, gastrointestinal tract, lymphoid organs, skeletal system, hematopoietic tissues and the reproductive organs can also be effected (Paterson and Lima, 2010). At higher toxic levels the birds show clinical signs of disease, mortality, nephritis (Elaroussi et al., 2006), hepatitis (Kumar et al., 2004). Ochratoxicosis developing from high degree of contamination of feeds with OTA is a common condition in different avian species. OTA induces degenerative changes and an increase in the weight of kidneys and liver (Stoev et al., 2011).

OTA is frequently present in poultry feeds and its ingredients (Saleemi et al., 2012). Its levels in the feeds are kept minimum by different methods including use of ingredients having low levels of OTA, proper storage and use of toxin binders to bind the preformed mycotoxins and rendering them unabsorbable from the gut. All these methods work with a variable degree of efficiency.
Ochratoxin A not only induces disease in chicken but also it may accumulate in the eggs and meat of the poultry and thus enters in human food chain. Therefore, all the efforts should be made to protect the commercial poultry birds from the toxic effects of ochratoxin. Another important strategy in case of clinical ochratoxicosis is to reduce the damage and losses by alleviating the deleterious effects of OTA by different hepato-protective and renal protective substances. At present different chemical substances and plant extracts are being used globally to treat the liver, kidney damage and immunosuppression caused by a variety of toxicants. Currently many hepatoprotective substances are being used to prevent liver damage due to these mycotoxins. Among these natural substances, silymarin is an important anti-toxin compound. Silymarin, an extract from seeds and fruits of *Silybum marianum*, is one of the most important natural hepato-renal protective substances containing a mixture of flavonoid isomers such as silibinin, isosilibinin, silidianin and silichristin (He et al., 2004). This plant is naturally present in northern areas of Pakistan including Kashmir. It is locally used by people as hepatoprotective substance in its crude form. In present study, purified SL in powdered form obtained from China was used. There is scanty information available on use of SL in poultry and livestock as antagonistic substance of mycotoxins. Therefore current study was planned to explicate the effects of SL in the cockerels to improve their health status in the presence of OTA in feed. However, no report is available about health improving effects of SL in cockerels in terms of their increased vigor to counter the injurious effects of OTA.

Vitamin E is an essential vitamin possessing an antioxidant activity. Vitamin E given at higher than recommended doses increase the number of antibody producing cells and raises antibody titer in chickens and mice (Hossain et al., 1998). Both VE and SL have an antioxidant effects and when given together may enhance the immunoprotective and immunostimulatory properties of each other (Horvath et al., 2001). Keeping in view the OTA induced pathological changes and hepato-protective effects of SL and VE in different species, the present project was designed to study OTA induced hematobiocological alterations in WL cockerels and protection provided by SL and VE.

**Materials and Methods**

**Birds feed and housing:** A total of 240, day-old, WL cockerels free from any clinical ailment were procured from a local hatchery and kept on rice husk litter material under standard housing and management conditions. Birds were fed on corn soybean based feed having 22% total protein and provided feed and water *ad libitum*.

**Experimental design:** After two days of acclimatization, these birds were divided into 12 groups from A to L having 20 birds in each group. The detailed layout of experiment is presented in Table 1. Duration of the experiment was 7 weeks. Ten birds from each group were sacrificed at 21 days of age and remaining birds at the end of experiment. Blood was collected with and without anticoagulant for hematological and serum biochemical studies.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Birds</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>Control</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>OTA 1 (1000 µg/kg feed)</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>OTA 2 (2000 µg/kg feed)</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>SL (3000 mg/kg feed)</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>VE (200 mg/kg feed)</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
<td>VE+SL</td>
</tr>
<tr>
<td>G</td>
<td>20</td>
<td>OTA 1+VE</td>
</tr>
<tr>
<td>H</td>
<td>20</td>
<td>OTA 2+VE</td>
</tr>
<tr>
<td>I</td>
<td>20</td>
<td>OTA 1+SL</td>
</tr>
<tr>
<td>J</td>
<td>20</td>
<td>OTA 2+SL</td>
</tr>
<tr>
<td>K</td>
<td>20</td>
<td>OTA +SL+VE</td>
</tr>
<tr>
<td>L</td>
<td>20</td>
<td>OTA 2+SL+VE</td>
</tr>
</tbody>
</table>

**Parameters studied:** Blood samples with EDTA were collected on the day of slaughtering and used for evaluation of following hematological studies. Erythrocyte counts were made with the help of hemocytometer using the technique described by Natt and Herrick (1952). Hemoglobin and PCV were determined according to method described by Sharaf et al. (2010). Serum total protein was determined by Biuret method (Oser, 1976). Serum albumin concentration was determined using commercially available kit of Diasis Diagnostic System, Turkey (DDS Lot # 8116). Serum alanine transferase was determined colorimetric method of Reitman and Frankel (1957) by using commercially available kit of Diasis Diagnostic System, Turkey (DDS Lot # 7275). Serum urea concentration was determined by using commercially available kit (DDS Lot # 7580).

**Statistical analysis:** Data thus obtained subjected to statistical analysis by using factorial test different means compared by using Duncan’s multiple range test (DMR). The level of significance was P≤0.05.

**RESULTS**

**Hematological parameters:** At 3rd week of experiment, all the hematological findings were non-significant, therefore, not present in the table. At week 7 of experiment erythrocyte count of group B (OTA1) and group C (OTA2) was significantly lower than the control group while of group G (OTA1+VE), group H (OTA2+VE), group I (OTA1+SL), group J (OTA2+SL), group K (OTA1+SL+VE) and group L (OTA2+SL+VE) was significantly different from control group. In groups D (SL), group E (VE) and group F (SL+VE), erythrocyte count was nonsignificantly different from the control group (Table 2).

At week 7 of experiment total leukocyte count of groups B, C, G, H, I, J and K was significantly higher than the control group. In groups D, E, F and L leukocyte count was non-significantly higher from the control group. At week 7 of experiment total leukocyte count of groups B, C, G, H, I, J and K was significantly higher than the control group. In groups D, E, F and L leukocyte count was non-significantly higher from the control group. At week 7 of experiment total leukocyte count of groups B, C, G, H, I, J and K was significantly higher than the control group. In groups D, E, F and L leukocyte count was non-significantly higher from the control group. At week 7 of experiment total leukocyte count of groups B, C, G, H, I, J and K was significantly higher than the control group. In groups D, E, F and L leukocyte count was non-significantly higher from the control group.
experiment Hb value of groups B, C, G, H, I, J, K and L was significantly lower than the control group while of groups D, E and F it differed non-significantly.

Table 2: Hematological parameters of cockerels administered various levels of ochratoxin A, Silymarin and Vitamin E at 49th day of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>RBC (10^6/µL)</th>
<th>WBC (10^6/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.76±0.47</td>
<td>28.00±0.60</td>
<td>2.70±0.08</td>
<td>24.53±0.32</td>
</tr>
<tr>
<td>B</td>
<td>9.11±0.32*</td>
<td>21.69±0.87</td>
<td>2.18±0.15*</td>
<td>27.16±2.27</td>
</tr>
<tr>
<td>C</td>
<td>8.50±0.37*</td>
<td>19.04±0.89*</td>
<td>1.91±0.17*</td>
<td>27.13±1.82</td>
</tr>
<tr>
<td>D</td>
<td>10.91±0.76</td>
<td>26.29±1.25</td>
<td>2.61±0.12</td>
<td>25.43±1.27</td>
</tr>
<tr>
<td>E</td>
<td>11.66±0.79</td>
<td>26.61±1.76</td>
<td>2.71±0.21</td>
<td>24.71±1.70</td>
</tr>
<tr>
<td>F</td>
<td>12.00±0.08</td>
<td>27.76±2.29</td>
<td>2.85±0.28</td>
<td>24.86±0.90</td>
</tr>
<tr>
<td>G</td>
<td>10.39±0.48*</td>
<td>24.17±1.29*</td>
<td>2.39±0.18*</td>
<td>26.79±0.81</td>
</tr>
<tr>
<td>H</td>
<td>10.11±0.56*</td>
<td>24.14±1.38*</td>
<td>2.35±0.14*</td>
<td>26.50±1.04</td>
</tr>
<tr>
<td>I</td>
<td>9.93±0.62*</td>
<td>23.76±1.50*</td>
<td>2.30±0.22*</td>
<td>27.57±1.72</td>
</tr>
<tr>
<td>J</td>
<td>9.20±1.00*</td>
<td>22.14±2.91*</td>
<td>2.24±0.33*</td>
<td>26.57±1.33</td>
</tr>
<tr>
<td>K</td>
<td>9.51±0.79*</td>
<td>24.39±1.24*</td>
<td>2.38±0.19*</td>
<td>26.43±1.33</td>
</tr>
<tr>
<td>L</td>
<td>9.89±0.82*</td>
<td>23.06±1.67*</td>
<td>2.26±0.23*</td>
<td>26.00±2.08</td>
</tr>
</tbody>
</table>

Values (Mean ± SD) in each column bearing asterisk differ statistically from control P<0.05.

Serum biochemical parameters: At 21 days of experiment total protein value of groups B and C was significantly lower than the control group. While in other groups (D to L) total protein value was non-significantly different from the control group. At 7th week of experiment groups treated with OTA1 and OTA2 (B and C), the total protein value was non-significantly lower than the control group while of other groups (D to L), it was non significantly different from the control group (Table 3).

At 3rd week of experiment in groups B, C, F, H, I and L albumin concentration was non-significantly lower than the control group. In group D, K, G and J the albumin concentration was non-significantly different from control group.

At 7th week of experiment in groups B, C treated with OTA1 and OTA2 respectively, and in groups G, I and K albumin concentration was significantly lower than the control group. In group D, E, F, L, H and J albumin concentration was non-significantly lower from the control group.

At 21 days of experiment in groups B, C, G, H, J, I and K was significantly higher than the control group. In groups D, E, and F creatinine concentration was non-significantly higher from the control group. In group L, it was significantly lower than the control group (Table 3).

At week 7 of experiment in group B, C, H, J, and group I, the creatinine concentration was significantly higher from the control group while in group K and group L the creatinine concentration was significantly lower than control group. In group D and E the creatinine concentration was non-significantly higher from the control group.

At week 3 of experiment, the ALT concentration in group B, C and H treated with OTA1, OTA2 and OTA2 + VE respectively was significantly higher than the control group while in groups J, I, K, L, G, D and E its concentration was non-significantly different from control group. At week 7 of experiment, ALT concentration in groups B, C, G and group H was significantly higher than the control group while of all other groups J, D, L, F, K, I, E was non-significantly higher from the control group.

At week 3 of experiment, urea concentration in groups B, C, G, H, J, K and L was significantly higher than the control group while in groups D, E, F and group I it was non-significantly higher from control group. At week 7 of experiment urea concentration in groups B, C, G, H, I and L was significantly higher than the control group while in groups D, E, F, I and J it was non-significantly higher than the control group.

Clinical signs and gross lesions: Birds treated with OTA1 (group B) and OTA2 (group C) were dull and depressed throughout the length of experiment. Birds died during the experiment and slaughtered at the day 21 and...
49 showed swollen liver and kidneys in OTA contaminated groups. Intestinal mucosa was congested in these OTA fed groups. It indicated pathological changes induced by OTA. In control, SL and VE groups birds were active and no such changes were observed on necropsy examination.

**DISCUSSION**

Keeping in mind this important issue the present study was designed to investigate toxicopathological effects of OTA in WL cockerels and their amelioration with silymarin and Vitamin E (α tocopherol). In present study, hematological parameters the RBCs count of groups B, C, G, H, I, J, K and L were significantly lower from control group while of groups D, E and F were non-significantly different from control group A. Similar results of decreased RBCs count in response to OTA feeding has been reported by different researchers (Mohiuddin et al., 1993; Stoev et al., 2011). Packed cell volume and laboratory - esponse to OTA feeding has been reported by different workers (Kalorey et al., 2006). This is related to OTA intoxication through feed.

In serum biochemical parameters total protein and albumin in group B (OTA1) and C (OTA2) were significantly lower from control group at day 21 and 49 of experiment. While in all the remaining groups total protein at day 21 and 49 was non-significantly different from control group. It is an important biochemical change induced by OTA in birds. But albumen of groups F, H, I and L were significantly lower from control group. Similar results have been reported by different workers (Hassan et al., 2010), where total protein and albumen decreased in a dose related manner and was minimum in higher OTA group (1.5 ppm) and maximum in control group. In present studies alanine amino-transferase (ALT) values of group B and C were significantly higher from control group, while all the remaining groups were non-significantly different from control group at day 21 of experiment. Sawale et al. (2009) and Hassan et al. (2010) have also been reported an increase in ALT concentration in dose related manner. Similarly at 49 day of experiment B, C and G were significantly higher from control group, while all the remaining groups were non-significantly different from control group at day 49 of experiment. Similar increasing trend in ALT values in OTA contaminated groups have also been reported by Wang et al. (2009) and Hassan et al. (2011). This increase in ALT level indicates acute hepatic necrosis. It is an important hepatotoxic effect of OTA.

In present study, creatinine values of groups B, C, G, H, J, I and K were significantly higher from control group at 21 and 49 days of experiment. Our findings of increased creatinine values coincide with the results previously reported in WL chicks and broilers (Kalorey et al., 2005; Sawale et al., 2009). In groups D, E and F (SL, VE and combination of both) these values were non-significantly different from control group. It indicates amelioration of adverse effects of OTA with these agents like SL and VE. Group L was non-significantly different from control group. Urea concentrations in present study in groups B, C, G, H, J, K and L significantly higher from control group, while of D, E, F (Silymarin, Vit. E and both in combination) and I were non-significantly different from control group at 21 days of experiment. At 49 days groups B, C, G and H were significantly higher from control group, while D, E, F, I and J non-significantly different from control group. Similar to our findings (Stoev et al., 2002; Koyinarski et al., 2007; Denli et al., 2008) reported significant higher serum uric acid concentration in OTA fed chickens.

The findings of present study may be concluded that birds kept on silymarin, VE and their combinations showed hemato-biochemical responses similar to those of control group. Birds fed SL, VE or their combination along with 1000 µg/OTA showed improvement in hemato-biochemical responses, almost similar to those of control birds suggesting a protective effect of SL against OTA. This protective effect, however, in birds fed SL, VE or their combination in birds fed 2000 µg/Kg OTA was partial. It indicates partial amelioration with these protective agents. These agents/substances may be used as protective agents in poultry to ameliorate the adverse effects of ochratoxin A.

**REFERENCES**


