Histopathological alterations of selenium toxicity induced in broiler (Birds)

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ABSTRACT

A study was conducted to determine the pathological toxic effect of selenium (Sodium selenite). A total number 120 one day old White Leghorn (WLH) healthy broiler birds were randomly divided into A, B and C groups. Each group contain 40 birds daily administration of sodium selenite @ 30ppm and @ 15 ppm in group A and B, respectively and group C was given plain drinking water daily for 42 days and kept as control. Macroscopically and microscopically revealed varying degrees of congestion and haemorrhages in lungs, liver, kidneys, heart and intestine in selenium treated birds. The bursa of Fabricius showed depletion of lymphoid cells between the interfollicular spaces. A significant (P<0.05) decrease in the haemoglobin and packed cell volume was noticed in both the selenium fed groups but the total erythrocyte count remain unchanged. Biochemical parameter revealed slight decline in their activity of serum ALT and AST and increased level of BUN and creatinine in group A and B as compared to group C, suggesting some degree of renal dysfunctioning.

Key words: Broiler birds, Selenium, Histopathology.

INTRODUCTION

Selenium originated with the discovery of selenium in 1818 by Swedish Chemist, J.J. Berzelins, from mining operation (Old-Fild, 1987), it is known for its dual nature and so its status in both animal and human nutrition is considered to be quite paradoxical. It is also known to be essential trace minerals for man and animal. Trace amounts of selenium in diet are required to prevent deficiency diseases such as exudative diathesis in birds, nutritional encephalomalacia, muscular dystrophy and pale bird syndrome. It has also been shown to enhance the immune response of chicks (Marsh et al., 1981). The occurrence of various deficiency diseases has led to the use of selenium supplements- sometimes in excess (overdosing) which may result in toxic for all animals. Overdosing might occur as a result of premix manufacturing errors or concurrent supplementation by several routes (Sandholm, 1993). Traditionally selenium has been added to poultry diets via inorganic source, such as sodium selenite. The toxicity of inorganic selenium, such as selenite, and selenate, has been heavily studied in animal including fish and aquatic birds. An assessment of toxicity of Se is complicated by its occurrence in many different chemical forms, some differing greatly in their toxicity to birds (Ganguli,2013). In view of the severe biological effect of selenium toxicity in all farm animals, a sequential toxicopathological study on this emerging problem has been conducted in broilers.

MATERIALS AND METHODS

A total number of 120 clinically healthy White Leg Horn (WLH) day old chicks of both the sexes were obtained from commercial hatchery and were reared on deep litter system of housing using rice husk with provision of artificial light. The birds were fed a standard commercial feed starter up to 14 days, thereafter a grower diet up to 28 days and finisher up to 42 days. Birds were allowed access to the diets and fresh and clean drinking water ad libitum. All the experimental birds were kept under close observation during entire period of study. Individually weighed birds were randomly divided in to 3 groups of 40 birds. Birds of group C was kept as untreated control and was given plain drinking water. Birds of group A and B were given treated drinking water with selenium @ 30 ppm and 15 ppm respectively from day first of experiment for 42 days.

To study the effect of selenium toxicity on the body weight gain, six randomly selected birds from each of the three groups were weighed individually at weekly intervals. All broiler birds died during the entire period of experiment and those sacrificed at the end of the experiment were thoroughly examined at necropsy. The presence of gross lesion in liver, kidneys, lungs, heart, intestine, bursa of Fabricius, skeletal muscles and brain were carefully recorded.

For Histopathology tissue samples of liver, kidney, lungs, brain and bursa of febricius were collected in 10 % neutral buffered formalin. The tissues were thoroughly...
washed in running tap water, dehydrated in ascending grades of alcohol; cleared in xylene and embedded in paraffin at 58°C. The paraffin embedded tissue sections of 4 to 5 µm were obtained and stained with haematoxylin and eosin (H & E) as per the method described by Bancroft and Stevens (2008). The stained sections were examined under light microscope and the lesions were recorded.

For haematobiochemical studies, blood samples (10 ml) from randomly selected three birds of each group were collected from wing vein at weekly intervals from day 7 to 28 days for haematological and biochemical studies. Four ml of blood sample with anticoagulant was used for estimation of haemoglobin (Hb; Sahli’s haemoglobinometer), packed cell volume (PCV); Schalm et al., (1986), total erythrocyte count and leucocyte count (TEC & TLC) Jain, (1993) and differential Leucocyte count (DLC) Leishman’s stain. The serum was formed from remaining six ml of blood samples utilized for estimation of aspartate transaminase and alanine transaminase (AST and ALT; Reitman and Frankel, 1957), alkaline phosphatase (ALP) Kind and King, 1954), total serum protein by biuret method (TSP), Wotton, 1974), serum creatinine (Folin and Wu, 1919) and blood urea nitrogen (BUN); Skeggs (1957).

The obtained data was analysed by analysis of variance and the significant result were shown by difference in superscripts with respective means (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

The toxic effect of sodium selenite in different doses on average body weight of birds was observed (Table 1). The average body weight of birds of group A and B showed significant (P<0.05) decrease in body weight gain as compared to birds of group C throughout the observation period of 42 days. The reduction in body weight was significantly (P<0.05) more in birds which were given higher concentration of sodium selenite (group A) then the birds of group B throughout the experiment. This finding was in accordance with the observations of earlier workers (EI-Begearmi and Combs, 1982 and Salyi et al., 1993) who have reported significant decline in body weight of selenium fed chickens. Failure to gain weight could be attributed to loss of appetite and decreased feed consumption. However, the possibilities of decrease in cellular metabolism in selenosis cannot be ruled out as Brown and de Wet (1962) reported that the selenium replaced sulphur in essential proteins and inhibited the enzymes concerned with tissue respiration.

Grossly selenium induced broilers exhibited dose dependent varying degrees of congestion and haemorrhages in liver, kidney, heart and intestine. But size of bursa of febricius showed marked enlargement. Broilers of control and low dose groups had no remarkable gross pathological changes. Histopathological changes in the sacrificed birds depended on the different dose of Se had mild congestion and moderate oedematous and mild vacuolar degenerative changes.

Liver: In group A by 7 days after sodium selenite administration revealed congestion of central veins and other blood vessels. The sinusoids were dilated and filled with blood and mild degenerative changes were noticed in the hepatocytes. Congestion of the central veins and other portal vessels was also seen at 14 days. Dilatation of sinusoids which were engorged with blood was more prominent, which caused pressure on the hepatic cords. The vascular changes persisted as above in the 21days. Congestion in sinusoids and necrosis in the parenchyma and connective tissue proliferation between the lobules are seen in the 28 days. (Fig.1).

Table 1: Average values (Mean ± SE) of body weight (g) changes in different groups of broiler chicken at different time intervals

<table>
<thead>
<tr>
<th>Days after selenium administration</th>
<th>Gr -A</th>
<th>Gr - B</th>
<th>Gr - C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>154.33 ± 5.19</td>
<td>153.22 ± 6.09</td>
<td>153.66 ± 6.29</td>
</tr>
<tr>
<td>7</td>
<td>155.87 ± 12.16</td>
<td>218.66 ± 15.27</td>
<td>326.00 ± 11.64</td>
</tr>
<tr>
<td>14</td>
<td>194.86 ± 9.24</td>
<td>389.66 ± 7.67</td>
<td>476.00 ± 22.66</td>
</tr>
<tr>
<td>21</td>
<td>206.33 ± 11.33</td>
<td>486.00 ± 25.65</td>
<td>639.00 ± 24.48</td>
</tr>
<tr>
<td>28</td>
<td>228.26 ± 9.98</td>
<td>536.33 ± 26.42</td>
<td>862.00 ± 25.65</td>
</tr>
<tr>
<td>35</td>
<td>258.22 ± 9.98</td>
<td>608.22 ± 34.16</td>
<td>1002.33 ± 21.81</td>
</tr>
<tr>
<td>42</td>
<td>All birds are dead</td>
<td>627.48 ± 26.42</td>
<td>1146.00 ± 39.17</td>
</tr>
</tbody>
</table>

Means within a column bearing different superscripts differ significantly (P<0.05). Values presented as Mean ± standard error.
The hepatic changes at 35 days of selenite feeding were similar to that observed at 28 days and the blood filled cavities were even more prominent in the 35 days. Similar changes of selenosis in liver has been reported by Sukra et al. (1976) in chick embryos and day old chicks.

Birds of group B revealed congestion and mild necrosis in parenchyma in 7th and 14th days of sodium selenite administration. Dilatation of hepatic sinusoids was the salient change present almost uniformly in the liver of birds examined on 21 days and onwards.

**Kidney:** Treated birds of group A had congestion and haemorrhages, degenerative and necrotic changes in renal tubules, distorted glomeruli and mononuclear cells infiltration were observed at 28 and 35 days of the experiment. (Fig.2). This observation is in agreement with Prasad et al. (1982). Similar histopathological alteration in kidneys were also reported earlier in Selenium treated chickens and mice respectively (Mirjana et al.2004 and Jacevic et al.2011).

**Lungs:** In groups A birds had varying degrees of pulmonary congestion and haemorrhages. But induced broiler of high dose group revealed oedematous changes, emphysema and proliferation of inter alveolar septa (Fig. 3). Present findings are goes along with findings of Salyi et al. (1993). The pulmonary changes of the present study were in accordance with the earlier findings of Jacevic et al. (2011) in Selenium intoxicated mice.

**Brain:** In birds of group A cerebellum showing degenerative of purkejee cells and congestion of blood vessels (Fig No. 4). The changes might be attributed to the acute toxicity of selenium and also due to the hypovolemic conditions. Brain revealed severely congested and dilated blood vessels along with haemorrhagic patches in the brain tissue of one chick that died on 21st day of selenium toxicity in group B.

**Bursa of fabricius:** Histopathological changes in the bursa of fabricius were in accordance with the findings of Salyi et al. (1993) who reported severe damage of the bursa of fabricius in broiler chicken experimentally 10 to 13 times overdosed with sodium selenite in the drinking water.

During present study selenium toxicity in broilers showed immunosuppressive effect on the basis of lower body weights and depletion of lymphoid population in bursa of fabricius.

**Haemato-biochemical observation:** A significant (p<0.05) decrease in TLC was observed in group A in comparison to group B and group C from 7 to 21 days post treatment (DPT). Though the mean values of TLC in group B were slightly lower than that of control but the difference was not significant except at 28 DPT. This observation was similar to finding of DAS et. al ( 1989 a) in guinea pigs. This leucopenia in selenium over dosed birds was due to decrease in number of both heterophils and lymphocytes.
Table 2: Effect of selenium treatment on haemato-biochemical values of broiler chicken at different days post treatment (DPT)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7DPT</td>
<td>14DPT</td>
<td>21DPT</td>
</tr>
<tr>
<td>Hb (g %)</td>
<td>7.94(^a)</td>
<td>6.52(^b)</td>
<td>7.86(^b)</td>
</tr>
<tr>
<td></td>
<td>±0.47</td>
<td>±0.27</td>
<td>±0.27</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>25.64(^a)</td>
<td>26.66(^a)</td>
<td>23.00(^b)</td>
</tr>
<tr>
<td></td>
<td>±1.33</td>
<td>±0.66</td>
<td>±1.15</td>
</tr>
<tr>
<td>TEC (x10(^6)/mm(^3))</td>
<td>2.98(^a)</td>
<td>2.73(^a)</td>
<td>2.60(^a)</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
<td>±0.05</td>
<td>±1.15</td>
</tr>
<tr>
<td>TLC (x10(^3)/mm(^3))</td>
<td>25.66(^b)</td>
<td>24.00(^b)</td>
<td>24.33(^b)</td>
</tr>
<tr>
<td></td>
<td>±1.76</td>
<td>±1.52</td>
<td>±1.02</td>
</tr>
<tr>
<td>AST (unit/dl)</td>
<td>102.00(^a)</td>
<td>92.83(^b)</td>
<td>81.33(^b)</td>
</tr>
<tr>
<td></td>
<td>±2.92</td>
<td>±8.08</td>
<td>±8.08</td>
</tr>
<tr>
<td>ALT (unit/dl)</td>
<td>24.00(^a)</td>
<td>27.00(^a)</td>
<td>29.66(^a)</td>
</tr>
<tr>
<td></td>
<td>±2.40</td>
<td>±4.21</td>
<td>±0.88</td>
</tr>
<tr>
<td>ALP (unit/dl)</td>
<td>46.68(^b)</td>
<td>41.66(^b)</td>
<td>38.33(^b)</td>
</tr>
<tr>
<td></td>
<td>±1.66</td>
<td>±1.66</td>
<td>±6.00</td>
</tr>
<tr>
<td>Total serum protein (g/dl)</td>
<td>5.53(^a)</td>
<td>5.23(^e)</td>
<td>4.80(^e)</td>
</tr>
<tr>
<td></td>
<td>±0.17</td>
<td>±0.46</td>
<td>±0.33</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>15.07(^a)</td>
<td>14.89(^a)</td>
<td>15.89(^b)</td>
</tr>
<tr>
<td></td>
<td>±1.20</td>
<td>±2.35</td>
<td>±1.13</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.72(^a)</td>
<td>2.28(^b)</td>
<td>2.45(^b)</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
<td>±0.37</td>
<td>±0.58</td>
</tr>
</tbody>
</table>
The result on bio-chemical studies revealed a moderate decrease in aspartate transaminase activity in birds of group A which was significant (P<0.05) on 14 and 21 DPT in comparison to group B and group C birds indicating hepatic damage in selenium overdosed birds. The mean alanine transaminase activity in the both the selenium fed groups birds was slightly lower than the control birds but the difference was not significant at any stage of the experiment. There was significant at any stage of the experiment. There was significant (P<0.05) reduction in the activity of alkaline phosphatase in the birds of group A on 7, 14 and 21 DPT. The birds of group B also had lower activity of this enzyme but difference was not significant except on 28 DPT (Table 2). The findings of decreased level of AST and ALT corroborated with the observation of Gessler (1965). Me Daniel et al. (1961) observed decrease in the alkaline phosphatase level in the fasting birds. Estimation of total serum proteins at different intervals showed a significant (P<0.05) reduction in their values on 14, 21 and 28 DPT in the birds of both the selenium fed groups (A and B) as compared to control birds indicating hypoproteinaemia in the selenium overdosed birds. The resultant hypoproteinaemia in the selenium treated birds was possibly because of its decreased synthesis by the degenerated and/or necrotic liver which was evident in necropsy finding. Similar finding were also reported by Das et al. (1989b) in the selenium treated birds. It was found that considerable elevation in the values of BUN in the birds of group A as compared to group B and C birds, but the difference were not significant. There was increase in serum creatinine level in group A birds but this increase in comparison to birds of group B and C was not significant except on 14 and 21 DPT. The mean value of creatinine level in group B birds was more than that in group C and difference was significant (P<0.05) after 2 weeks of selenium feeding. Elevation in the levels of these metabolites is suggestive of some degree of renal damage in the birds fed with Selenium. Ahmed et al. (1988) has reported elevation in the level of blood urea in goat suffering from Selenosis. Increase in serum creatinine in high does selenium fed birds might be due to nephrotoxic action of sodium selenite, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage (Mohmadsadik et al. 2000)

REFERENCES


