Effect of acute fluoride intoxication on some hematological changes in chicken (*Gallus domesticus*).

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ABSTRACT

A very small amount of fluoride is beneficial for human; whereas overdosing cause problems like neural disorders, memory loss and deficit in intelligence in human as well as other mammals. Present study was designed to look into the hematological changes by acute fluoride intoxication in *Gallus domesticus* (domestic chicken). The birds were divided into four treatment groups: group A acted as control and three groups as B, C & D injected with NaF at 10, 20 and 30 µg/g of body weight, respectively. The birds were sacrificed and blood was collected for hematological studies. It was observed that there was significant decrease (P < 0.05) in Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC) and hemoglobin content in groups C and D. The results showed that birds were anemic after the exposure to acute dose of NaF. It was also observed that bones were deformed leading to lesser production of bone marrow, a site for blood formation, which is the reason for severe decrease in hematological values.

Key words: NaF, Hematology, Chicken, Acute Intoxication, Bone Deformation.

INTRODUCTION

Any combination of elements containing fluorine ion is fluoride. In high concentrations, high concentrations of fluoride salts are toxic to skin or eye. The lethal dose of most common salt of fluoride i.e. sodium fluoride (NaF) for most adult humans is 5 to 10 g (Gosselin et al., 1984 and Baselet, 2008). The fluorine was not produced commercially before the Second World War, the use of fluorine in the processing of uranium ores promoted its manufacture (International Programme on Chemical Safety, 2002).

After the absorption of NaF from intestine, a big part of the fluoride is rapidly distributed to skeletal system and small proportion in the teeth. In skeletal system it promotes the development of bone marrow; whereas, in teeth gives protection from caries (Schmidt & Leuschke, 1990). Studies have shown that high levels of fluoride are accumulated in the brain causing neurologic disorders, motor disruption, intelligence deficit and learning disabilities in human (Li et al., 1995). Fluoride overdose in rabbits caused corneal epithelial defects and necrotic area in the conjunctive of the rabbits. Furthermore, lameness was observed in ungulates due to fluoride accumulation in their skeleton. Fluoride accumulation also creates premature mortalities and dental lesions in wild animals (Phyllis, 1995).
Excessive fluoride contamination in drinking water is common in developing countries like India, China, Uzbekistan, Ethiopia and Pakistan. Many minerals contain soluble fluoride, when the ground water passes through such fluoride bearing rock formations; the water becomes contaminated (Abida et al., 2007).

In Pakistan, during year 2000, bone deformity disease was reported in districts; Kalanwala and Kasur of Punjab. The investigations showed that disease was due to high levels of fluoride in the drinking water affecting extremities of legs, vertebral column and the skull bones. The present study was aimed to evaluate the effects of acute fluoride intoxication on different blood parameters as in domestic chicken, Gallus domesticus. In the affected area the poultry birds were also given drinking tap water containing the high levels of fluoride. The study was focused to determine the risks of NaF intoxication in poultry as model organism and validation of results obtained on other organisms.

MATERIALS AND METHODS

Eighty domestic chickens were used in the study. One day old chicks were purchased from local market. Before starting the experiment, birds were raised and acclimatized for a period of two weeks in Animal House at GC University, Lahore. After two weeks animals were divided into four groups of 20 birds each.

Group A: This group was kept as control, fed on normal feed and was not exposed to any toxicant. Five chicks were sacrificed each day and the hematological values were used as a reference to investigate the NaF intoxication effects in the treatment groups.

Group B: This group received normal feed and each bird was injected with NaF 10 µg/g body weight. First day all the chicks were injected. On second day, five chicks were sacrificed for hematological studies. Remaining 15 chicks were again exposed to the same dose. Five chicks were sacrificed each after one, two, three and four days with the exposure of 10, 20, 30 & 40 µg /g body weight of NaF, respectively.

Group C: This group was given normal feed and each bird was injected with NaF 20 µg/g body weight. First day all the chicks were injected. On second day, five chicks were sacrifices for hematological and serological studies. Remaining 15 chicks were again exposed to the same dose. Five chicks were sacrificed each after one, two, three and four days with the exposure of 20, 40, 60 & 80 µg /g body weight of NaF, respectively.

Group D: This group was given normal feed & was injected with 30 µg/g body weight of NaF. First day all the chicks were injected. On second day, five chicks were sacrificed for hematological and Serological studies. Remaining 15 chicks were again exposed to the same dose. Five chicks were sacrificed each after one, two, three and four days with the exposure of 30, 60, 90 & 120 µg /g body weight of NaF, respectively.

Hematological studies were carried out by using improved nebular Hemacytometer (Bright-Line™). The quantitative data are represented as mean + S.D (Standard Deviation). Statistical package for social sciences (SPSS-13)
software was used for statistical analysis. Hypothesis testing methods included the one way analysis of variance (ANOVA). P values less than 0.05 were considered to indicate the statistical significance. (Sokal & Rohlf, 1995; Kumar et al., 2006).

RESULTS AND DISCUSSION

The erythrocytes of the birds are nucleated and oval-shaped. The colour of individual cell is yellowish green due to presence of hemoglobin content. The red blood cell count per millimeter of the mature chicken varies from $2.74 \times 10^9$ to $3.2 \times 10^9$ (Susheela & Jain, 1983). In present study, erythrocyte count in control group was recorded as $2.76 \times 10^9 \pm 0.01$. In the Group B, RBC counts after first dose was $2.74 \times 10^9 \pm 0.02$. The erythrocyte count decreased after second, third and fourth dosing to $2.73 \times 10^9 \pm 0.02$, $2.71 \times 10^9 \pm 0.03$, and $2.72 \times 10^9 \pm 0.32$, respectively (Table 1). In Group C, there was drastic decrease in RBC count after first, second, and third dose to $2.73 \times 10^9 \pm 0.02$, $2.72 \times 10^9 \pm 0.25$ and $2.71 \times 10^9 \pm 0.02$ respectively (Table 1). The birds exposed to four doses of NaF showed severe anemia and their RBC count decreased to $2.68 \times 10^9 \pm 0.03$ (Table 1). In Group D, the condition was too severe especially with the birds that received four doses of toxicant. The RBC values recorded were: $2.73 \times 10^9 \pm 0.02$, $2.69 \times 10^9 \pm 0.03$, $2.66 \times 10^9 \pm 0.04$ and $2.63 \times 10^9 \pm 0.03$, after receiving one, two, three and four doses of the toxicant, respectively as shown in Table 1.

Table 1: Effect of NaF Intoxication on Total Erythrocyte Count (TEC)

<table>
<thead>
<tr>
<th>Dose received</th>
<th>TEC. in group A ($\times 10^9$)</th>
<th>TEC. in group B ($\times 10^9$)</th>
<th>TEC. in group C ($\times 10^9$)</th>
<th>TEC. in group D ($\times 10^9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.76 \pm 0.01$</td>
<td>$2.74 \pm 0.02$</td>
<td>$2.73 \pm 0.02$</td>
<td>$2.73 \pm 0.02$</td>
</tr>
<tr>
<td>2</td>
<td>$2.76 \pm 0.01$</td>
<td>$2.73 \pm 0.02$</td>
<td>$2.73 \pm 0.25^*$</td>
<td>$2.69 \pm 0.03^*$</td>
</tr>
<tr>
<td>3</td>
<td>$2.76 \pm 0.01$</td>
<td>$2.71 \pm 0.03^*$</td>
<td>$2.71 \pm 0.02^*$</td>
<td>$2.66 \pm 0.04^*$</td>
</tr>
<tr>
<td>4</td>
<td>$2.76 \pm 0.01$</td>
<td>$2.72 \pm 0.32^*$</td>
<td>$2.68 \pm 0.03^*$</td>
<td>$2.63 \pm 0.03^*$</td>
</tr>
</tbody>
</table>

*indicates the significant results (P < 0.05)

The erythrocyte count may vary due to the hemolysis of R.B.Cs or destruction of erythropoietic organs. Anjum & Shakoori (1993) also reported anemia induced by fluoride intoxication.

In birds the average leukocyte count is $13.5 \times 10^6$/ml. Different studies have shown that Total Leukocyte Count (TLC) reduced when the individual birds were exposed to different intoxicants. Al. Khel et al., (1998) observed the reduction in leukocyte after the exposure of Oreochromis niloticus to different sub-lethal concentrations of Manganese.

In the present study the TLC values decreased. In the control group TLC was recorded as $13.62 \pm 0.24 \times 10^6$. In group B, the TLC was recorded almost same as in the control group indicating that the birds were able to cope with dosage of fluoride intoxication; whereas, the birds exposed to four doses of NaF showed the $13.04 \pm 0.25 \times 10^6$. In group C, reduction in TLC was more pronounced especially in birds receiving three and four doses of toxicant. Total
leukocytic count were as; 13.04± 0.25 × 10^6 and 13.02 ± 0.18 × 10^6 respectively (Table 2). The TLC values were adversely reduced in group D, 13.14± 0.26 × 10^6, 12.90± 0.14 × 10^6, 12.85± 0.24 × 10^6 and 12.78± 0.14 × 10^6 were the TLC values receiving one, two, three and four doses of NaF.

**Table 2: Effect of NaF Intoxication on Total Leukocyte Count (× 10^6)**

<table>
<thead>
<tr>
<th>Dose received</th>
<th>TLC in group A (× 10^6)</th>
<th>TLC in group B (× 10^6)</th>
<th>TLC in group C (× 10^6)</th>
<th>TLC in group D (× 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.62± 0.24</td>
<td>13.60± 0.30</td>
<td>13.26± 0.33*</td>
<td>13.14± 0.26*</td>
</tr>
<tr>
<td>2</td>
<td>13.62± 0.24</td>
<td>13.54± 0.24</td>
<td>13.20± 0.30*</td>
<td>12.90± 0.14*</td>
</tr>
<tr>
<td>3</td>
<td>13.62± 0.24</td>
<td>13.34± 0.37*</td>
<td>13.04± 0.25*</td>
<td>12.85± 0.24*</td>
</tr>
<tr>
<td>4</td>
<td>13.62± 0.24</td>
<td>13.04± 0.25*</td>
<td>13.02± 0.18*</td>
<td>12.78± 0.14*</td>
</tr>
</tbody>
</table>

*indicates the significant results (P < 0.05)

The same findings were also reported by Hashmi (1999), during mercury intoxication in _Labeo rohita_. The leucopoiesis was impaired by fluoride in the present study.

Hemoglobin is an important protein which supplies the oxygen to the body of animals. The concentration of Hemoglobin in chicken varies from 7- 8.9 (g/dl). The avian hemoglobin contains the four heme units just like the mammals (Ali, 1990). The hemoglobin concentration in group A (control) was 7.96± 0.20. In group B, there was a decrease in hemoglobin in response to dose of the toxicant. Following were the hemoglobin concentrations; 7.84± 0.26, 7.74± 0.21, 7.48± 0.32 and 7.38± 0.25 recorded after one, two, three and four doses (Table 3). In Group C, the birds showed remarkable decrease in hemoglobin concentration as the leukocytes counts were 7.50± 0.30, 7.43± 0.30, 7.36± 0.18 and 7.12± 0.28 after the one, two, three and four doses respectively. In group D, there was a drastic decrease in hemoglobin concentration, the birds after first dose of the toxicant showed 7.46± 0.30 of hemoglobin while the birds that received four doses in this group had 6.68 ± 0.26 (g/dl) of hemoglobin.

**Table 3: Effect of NaF Intoxication on Haemoglobin Concentration. (g/dl).**

<table>
<thead>
<tr>
<th>Dose received</th>
<th>Hb Conc. in group A (g/dl)</th>
<th>Hb Conc. in group B (g/dl)</th>
<th>Hb. Conc. in group C (g/dl)</th>
<th>Hb Conc. in group D (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.96± 0.20</td>
<td>7.84± 0.26</td>
<td>7.50± 0.30*</td>
<td>7.46± 0.30*</td>
</tr>
<tr>
<td>2</td>
<td>7.96± 0.20</td>
<td>7.74± 0.21*</td>
<td>7.44± 0.30*</td>
<td>7.38± 0.23*</td>
</tr>
<tr>
<td>3</td>
<td>7.96± 0.20</td>
<td>7.48± 0.32*</td>
<td>7.36± 0.18*</td>
<td>7.12± 0.28*</td>
</tr>
<tr>
<td>4</td>
<td>7.96± 0.20</td>
<td>7.36± 0.25*</td>
<td>7.12± 0.28*</td>
<td>6.68 ± 0.26*</td>
</tr>
</tbody>
</table>

*indicates the significant results (P < 0.05).

The possible reasons for the decrease in hemoglobin concentration were low level of RBC production and hemolysis. The bones of these chicks were deformed due to fluoride intoxication resulting in decrease of bone marrow production which is a site for RBC production, leading to anemia and low hemoglobin concentration.
REFERENCES


