Effect of Sub Lethal Doses of Thiamethoxam (A Pesticide) on Hemato-Biochemical Values in Cockerels

Shafia Tehseen Gul1, Ahrar Khan1*, Muhammad Farooq1, Shoaib Niaz1, Maqbool Ahmad2, Aisha Khatoon1, Riaz Hussain1, Muhammad Kashif Saleemi3 and Mohammad Faroque Hassan4

1Department of Pathology; 2Department of Therienology, University of Agriculture, Faisalabad-38040; Pakistan
3University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
4Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan
*Corresponding author: ahrar1122@uaf.edu.pk

A B S T R A C T

The objective of the current study was to find out the toxic effects of sub lethal doses of thiamethoxam on adult poultry birds. For this purpose, a total of 40 cockerels having an age of about 14 weeks were procured from local market and divided into five equal groups. Birds were kept in wire cages under standard management conditions. Birds were given Thiamethoxam @ 250, 500, 750 and 1000mg/kg BW to group A, B, C and D, respectively. Group E served as a control. Blood and serum samples were collected at 15th and 30th day of experiment and analyzed for the various hematological (TEC, TLC, Hb and PCV) and biochemical parameters (total proteins, albumin, globulin, creatinine, blood urea, ALT and AST). The data thus collected were subjected to ANOVA through M-Stat software. Results thus obtained indicated that the sub lethal doses of thiamethoxam reduced significantly (P≤0.05) the hematological values including TEC, Hb, PCV and TLC in a dose dependant manner. The biochemical parameters like total proteins, albumin and globulin were significantly affected by the thiamethoxam. As the dose of the thiamethoxam was increased, these parameters decreased significantly as compared to control group. On the other hand, the values of enzymes ALT and AST were significantly higher in treated groups.

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INTRODUCTION

Insecticides/pesticides are the biological or chemical agents used in agriculture to destruct the pest population those are harmful to the crops. In veterinary, these are used for the control of external parasites in animal and poultry houses. But they are also a source of environmental and food contamination which ultimately affects the human population. Different types of pesticides are used among these neonicotinoids (including imidacloprid, acetamiprid and thiamethoxam) share 25% in the global insecticide market, are particularly used to control the sucking pests on various seasonal crops (Jeschke et al., 2011; Swenson and Casida, 2013).

Thiamethoxam (TMX), synthesized in 1991 and initially considered as produg most widely used throughout the world due to its supposed selectivity (Shalaby et al., 2010; Thany, 2010; Yan et al., 2016). TMX has adverse effects on the nicotinic acetylcholine receptors (nAChR) by copying the chemical messenger of acetylcholine and binding to the specific receptor site, ultimately blocking the nicotinic receptor. Due to this action, the insect is paralyzed and ultimately died (Green et al., 2005). Initially, TMX was considered the safest option but laterally various environmental adverse effects were reported due to which it was banned in the UK in 2003 till further studies (European Commission, 2013; Laycock et al., 2014).

Common uses of TMX are for seed dressing and spraying on major crops, including brinjal, citrus, cotton, corn, mango, maize, wheat, barley and sorghum etc. those are major components of the food chain. The pesticides pass through various ecosystems and enter into the food, water and air due to its some unique characteristics like it is non-volatile, hydrolysis stable at neutral and acidic pH, low soil sorption, bioaccumulation and low degradation,
et al., 2012; Morrissey et al., 2015; Ugrulu et al., 2015). This environmental and food contamination in the form of residues poses a serious threat to the non-target species and public health (Ugrulu et al., 2015; Yan et al., 2016).

Adverse effects of thiamethoxam have been reported on hematological, biochemical and behavioral parameters in laboratory animals due to oral/intraperitoneal administration of TMX including reduced numbers of erythrocytes and leukocytes, decreased hemoglobin and hematocrit values. Immunosuppressive effects of TMX have also been reported (Mason et al., 2013; Sinha and Thaker, 2014). In poultry, chronic exposure to the thiamethoxam results in a decrease in hatching time, reduced egg shell production or egg shell thinning (Bharadwaj et al., 2010; Ganguly, 2013).

In Pakistan, TMX and Imidacloprid are being used for the control of the pests like *Bemisia tabaci* in the developmental stages of various crops like cotton, wheat, barley, sorghum, canola and corn. Among these crops most of these crops are being used in terms of meals in poultry feed which poses a toxic threat to the birds in terms of residues (Naveed et al., 2010; Khan et al., 2015). Keeping in view the use of TMX on major crops this project was designed to find out the effects of sub-lethal doses of TMX on hematobiochemical parameters of cockerels. Previously, no work has been performed on toxic effects of thiamethoxam in cockerels.

**Materials and Methods**

Experimental birds and treatment: A total of 40 cockerels, having 14 weeks age were procured from local market and maintained under similar management and standard housing conditions. The room temperature was maintained at 22-26°C with a relative humidity (60-80%). The birds were provided basal diet and clean drinking water around the clock. The birds were divided into five equal groups (eight birds/group) and housed in wire cages and the trial continued for 30 days. Group A, B, C and D were given the thiamethoxam treatments @ 250, 500 and 750mg/kg BW, respectively daily through a crop tube following the prescribed dose calculation strategy by Kumar et al. (2010). Group E was kept as a control. Blood and sera were collected by following the standard protocols at the 15th and 30th day of the experiment. This research plan was approved by Graduate Studies and Research Board, University of Agriculture, Faisalabad, Pakistan.

Parameters studied: Hematological parameters including total erythrocyte counts (TEC) and total leucocyte counts (TLC), hemoglobin and hematocrit values were measured by following the procedures described by Sharaf et al. (2013). All the biochemical parameters were determined by commercial Kits (Merck, France) including serum total proteins (Cat# 5.17630.0001), albumin (Cat# 5.17620.0001), alanine aminotransferase (ALT) (Cat# 5.17531.0001), aspartate aminotransferase (AST) (Cat# 5.17521.0001), creatinine (Cat# 5.17550.0001) and blood urea (Cat# 5.17611.0001). The amount of globulin was determined by subtracting the value of albumin from total proteins.

Data analysis: The data obtained from the above experiment were subjected to factorial statistical analysis using ANOVA and different group means were compared by Duncan’s multiple range tests using M-stat statistical software package. The level of significance was P≤0.005.

**RESULTS**

Hematology: Different blood parameters of cockerels treated with different doses of thiamethoxam were measured at the day 15th and 30th of the experiment. Red blood cells (10^6/L), white blood cells (10^9/L), packed cell volume (%) and hemoglobin concentrations (g/dl) of different groups were measured by using appropriate methods. At day 15th, Total erythrocyte counts (TEC) were highest in low dose group A, followed by B and D, while the lowest TEC were evident in group C. The difference among treatment groups A and B and C and D was statistically significant. However, TEC were lower in all treated groups as compared to the control group and this difference was statistically significant (P≤0.05). The response of erythrocyte was in a dose dependant manner (Table 1).

The other parameters like Hb and hematocrit values determined at 15th and 30th days showed a similar trend to that of total erythrocyte counts. Lowest Hb and hematocrit values were recorded in high dose group D and it was significantly different (P<0.05) from that of control group, even the difference among all the treatment groups was also significant (Table 1).

The leucocytes have a different trend to that of erythrocytes. Leukocytes were higher in the treatment groups as compared to the control groups and this difference was statistically significant (P<0.05) at 30th day of the experiment. Similarly, the difference among different treatment groups was also statistically significant (Table 1).

Biochemical parameters: All the biochemical parameters were determined by commercial kits as per instructions of the manufacturers. At 15th and 30th days, total protein values were significantly lower in the treated groups as compared to the control group. At 15th day among the treated groups A and B, and similarly B and C, the difference was statistically non-significant. However, it was significant among C and D groups. However, at 30th day, the difference was statistically non-significant among all the treated groups (Table 2).

At both 15th and 30th days, albumin quantity was highest in low dose group (group A), and increasing trend was observed with the increase in the dose of the toxin and this difference between the treatment groups was statistically non-significant. However, albumin was significantly lower than that of the control group (group E). A similar trend was shown by the globulin quantity at both the experimental days (Table 2).

Creatinine values were decreased gradually as the dose of the toxin was increased and it was significantly higher than that of control group E, at both 15th and 30th days of the experiment. But in case of blood urea, opposite trend was observed. The blood urea level was significantly higher in the control group as compared to the treatment groups (A, B, C and D) and the lowest value was observed in group D, receiving the highest dose of the toxin. The difference among the treatment groups was statistically non-significant (Table 2).
Serum alanine aminotransferase (ALT) quantity was the highest in group D (treated with high dose of the toxin) and decreased gradually in a dose dependent manner. The ALT values of group C and D were significantly higher than that of the control group at both 15th and 30th days of the experiment. A similar trend was observed in case of AST quantity in serum (Table 2).

**DISCUSSION**

Various insecticides and pesticides are used for the control of ectoparasites in agriculture and veterinary practices which results in decreased productivity. Thiamethoxam was considered initially as the safest choice and mostly used for the control of house fly and other ectoparasites in animal and poultry houses. In agriculture, it is used to control the pests and seed treatment of various major crops like maize, corn, wheat, cotton etc. (Mondal et al., 2009; 2014). But now-a-days has been banned in European countries, however, it is extensively used in Pakistan in the form of sprays and seed treatments. So, poultry birds come in contact with this pesticide in various forms. This experiment was conducted to evaluate the toxicity of sub lethal doses of TMX in cockerels.

Hematological parameters including TEC, PCV and HB were significantly decreased with the increase in dose rate of TMX. This trend was observed throughout the experiment. Previously, similar results have been reported due to the exposure to other chemicals even at therapeutic level, including endosulfan (Chaudhary and Joshi, 2002), Withania somenifera (Mishra et al., 2005), metasystox (Sasikala et al., 2011) and due to thiamethoxam exposure in Oreochromis niloticus (Trewavas) (Roy and Nath, 2011). This might be due the effect of pesticide/chemical toxicity in the hemopoietin system (Mishra et al., 2005; Kumar et al., 2010; Roy and Nath, 2011). Blood values are an important indicative for the diagnosis of drug or chemical induced anemia. This anemia could be due to the destruction of the erythrocytes that is induced by the thiamethoxam influx into the RBCs (Roy and Nath, 2011). Due to the decrease in the number of erythrocytes, ultimately the levels of hemoglobin and hematocrit become lower.

In the present study, different biochemical parameters including, total proteins, albumin and globulin were significantly affected by the thiamethoxam. As the dose of the thiamethoxam was increased, these parameters significantly decreased as compared to control group. These findings were consistent to the findings of Siddique et al. (2007), who reported the similar trend of these parameters due to imidacloprid toxicity, the insecticide of the same group.

The other parameters including creatinine, blood urea, alanine aminotransferase and aspartate aminotransferase were significantly increased with the increasing dose of the TMX and were also higher from that of the control group. This increase has been also reported previously by Kammon et al. (2010) due to exposure to some other pesticides, including chlorpyrifos and imidacloprid in layer chicks. Serum enzymes (alanine aminotransferase and aspartate aminotransferase) are most commonly used as diagnostic markers of hepatic injury. In most of the insecticides toxicities, the liver is damaged, particularly

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**Table 1**: Hematological parameters of cockerels treated with Thiamethoxam at different doses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC (10^9/L)</td>
<td>11.43±0.89</td>
<td>12.38±1.30</td>
<td>12.75±1.28</td>
<td>12.88±1.55</td>
<td>12.88±1.46</td>
</tr>
<tr>
<td>TLC (10^9/L)</td>
<td>45.20±3.12</td>
<td>40.00±6.06</td>
<td>38.21±0.16</td>
<td>37.30±1.06</td>
<td>39.67±0.83</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>47.62±1.06</td>
<td>35.25±6.7b</td>
<td>35.75±5.3b</td>
<td>35.75±5.3b</td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>47.62±1.06</td>
<td>35.25±6.7b</td>
<td>35.75±5.3b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**: Biochemical parameters of cockerels treated with Thiamethoxam at different doses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Proteins (g/dl)</td>
<td>2.26±0.06b</td>
<td>2.00±0.07c</td>
<td>1.85±0.03d</td>
<td>1.71±0.06e</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>7.11±0.30b</td>
<td>6.56±0.65c</td>
<td>6.31±0.67cd</td>
<td>5.75±0.33d</td>
<td></td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>10.78±0.39b</td>
<td>10.34±0.60b</td>
<td>9.77±0.24c</td>
<td>9.49±0.32c</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.47±0.07a</td>
<td>10.00±0.93a</td>
<td>12.87±1.13a</td>
<td>11.43±0.60a</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>2.47±0.07a</td>
<td>10.00±0.93a</td>
<td>12.87±1.13a</td>
<td>11.43±0.60a</td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>2.43±0.21a</td>
<td>10.87±1.04a</td>
<td>11.43±0.60a</td>
<td>4.03±1.69a</td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>2.43±0.21a</td>
<td>10.87±1.04a</td>
<td>11.43±0.60a</td>
<td>4.03±1.69a</td>
<td></td>
</tr>
</tbody>
</table>

Values (mean±SD) in each column followed by different letters differ significantly (P≤0.05) at a specific experiment day.
hepatocytes, due to this damage to the hepatocytes membrane, the enzymes, including ALT and AST are drained into the blood and hence the increased serum level is observed (Kammon et al., 2010; Shalaby et al., 2010; Adejumo et al., 2015). The creatinine is synthesized in the liver and then enters the blood circulation, from where it goes to the muscles, so if there is extensive liver damage, ultimately the serum creatinine level is also increased (Kumar et al., 2010). Similarly, increased urea level is attributed to the renal damage or failure. The increase in serum urea is due to the conversion of toxic ammonia into non-toxic urea (Kumari et al., 2006; Kumar et al., 2010).

Conclusions: It was concluded that thiamethoxam causes toxicity even at sub lethal doses which ultimately results in various hematological and biochemical changes in cockerels. If the use of this pesticide continues to treat the crops, it will ultimately affect the end users not only the poultry birds but also the human beings. Therefore, there is a dire need to find out the alternatives to these pesticides which are non-toxic to the environment.

Authors’ contribution: STG, AK and RH conceived the idea and designed the experiment. MA, MKS, AK and MFH were involved in data analysis, interpretation and write up of the manuscript. All authors approved the manuscript.

REFERENCES


