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RESEARCH ARTICLE

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

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ABSTRACT

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 Thaimethoxam @ 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 ($P \leq 0.05$) 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 neonictinoids (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).

Thaimethoxam (TMX), synthesized in 1991 and initially considered as prodrug 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,

etc. (Zhao *et al.,* 2012; Morrissey *et al.,* 2015; Ugurlu *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 (Ugurlu *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 thaimathoxam results an increase 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 hemato-biochemical 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 form 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, Faislabad, Pakistan.

Parameters studied: Hematological parameters including total erythrocyte counts (TEC) and total leukocyte 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# 5.17531.001), aspartate aminotransferase (AST) (Cat# 5.17521.001), creatinine (Cat# 5.17550.0001) and blood urea (Cat# 5.17611.001). 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 $P \leq 0.005$.

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^{12}/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 ($P \leq 0.05$). 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 leukocytes 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).

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

Groups	TEC (10^{12}/L)	TLC (10^{9}/L)	Hb (g/dl)	PCV (%)
At 15th day of Experiment				
A	2.05±0.09b	12.38±1.30a	11.43±0.89a	42.50±3.12a
B	1.86±0.07c	12.75±1.28a	10.12±1.06b	40.00±1.60b
C	1.62±0.04d	12.88±1.55a	9.86±0.83b	38.37±1.06c
D	1.65±0.04d	12.88±1.46a	9.78±0.74c	34.87±0.83c
Control	2.47±0.07a	10.00±0.93b	12.87±1.13a	47.62±1.06d
At 30th day of Experiment				
A	2.26±0.06b	7.71±0.30b	10.78±0.39b	35.25±6.67b
B	2.00±0.07c	6.56±0.65c	10.34±0.60b	35.75±1.83b
C	1.85±0.03d	6.31±0.67cd	9.77±0.24c	34.50±2.45b
D	1.71±0.06e	5.75±0.33d	9.49±0.32c	34.87±3.04b
Control (E)	2.43±0.21a	10.87±1.04a	11.43±0.60a	40.63±1.69a

Values (mean±SD) in each column followed by different letters differ significantly (P≤0.05) at a specific experiment day.

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

Parameters	Units	Experimental Groups				
		A	B	C	D	Control (E)
At 15th day of Experiment						
Total Proteins	g/dl	4.26±0.45b	4.22±0.38bc	3.81±0.23cd	3.45±0.59d	6.17±0.15a
Albumin	g/dl	2.35±0.05b	2.31±0.09b	2.58±0.22b	1.95±0.18c	4.30±0.37a
Globulin	g/dl	1.91±0.49a	1.91±0.35a	1.23±0.41a	1.50±0.53a	1.87±0.50a
Creatinine	mg/dl	3.24±0.64a	3.46±0.48a	3.66±0.17a	3.19±0.41a	1.90±0.92b
Urea	mg/dl	1.54±0.82b	1.25±0.65b	0.77±0.86b	1.03±0.34b	2.58±0.50a
ALT	IU/L	6.27±1.28c	6.87±0.06c	8.69±0.72b	11.99±1.92a	7.03±0.85c
AST	IU/L	233±24.6c	284.5±19.1b	314±13.1a	322±18.01a	192.5±7.9d
At 30th day of Experiment						
Total Proteins	g/dl	4.21±0.21b	3.96±0.04c	3.55±0.09d	3.15±0.11e	6.25±0.17a
Albumin	g/dl	2.54±0.12b	2.44±0.17b	2.12±0.13c	1.84±0.10d	4.00±0.27a
Globulin	g/dl	1.67±0.32b	1.52±0.17bc	1.42±0.13bc	1.30±0.12c	2.25±0.23a
Creatinine	mg/dl	3.45±0.17b	3.74±0.10ab	4.01±0.08a	4.06±0.15a	1.76±0.49c
Urea	mg/dl	1.03±0.23c	1.32±0.08b	1.51±0.11ab	1.58±0.14a	0.94±0.11c
ALT	IU/L	10.29±.67b	11.89±0.25b	14.79±1.95a	16.52±2.74a	6.98±0.61c
AST	IU/L	226±15.38c	294.5±21.1b	308±65.9ab	340.2±31.8a	191.7±14.7c

Values (mean±SD) in each column followed by different letters differ significantly (P≤0.05) at a specific experiment day.

Serum alanine aminotransferase (ALT) quantity was the highest in group D (treated with high dose of the toxin) and decreased gradually in a dose dependant 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 being 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 (Chauhdary 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

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 creatnine 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 thaimethoxam 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 project. MF and SN executed the experiment. MA, MKS, AK and MFH were involved in data analysis, interpretation and write up of the manuscript. All authors approved the manuscript.

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