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Original Research Paper

Haematological Study on the Fungicide Ziram Induced Acute, Subacute and Subchronic Toxicity in Broiler Chickens

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ABSTRACT

The present work was conducted to elucidate the haematological changes in broiler chickens after Ziram intoxication. For this study, 150 apparently healthy broiler chicks were reared up to 2 weeks of age and randomly allocated to four groups, i.e. acute (N=10), sub-acute (N=20), sub-chronic (N=90) and control (N=30). Toxicity was induced following a single oral dose of Ziram @ 100mg/kg body weight for acute intoxication; 5 mg/kg body weight per day for 20 days in case of sub-acute group and 1mg/kg body weight per day for the study period of 90 days in case of sub-chronic intoxication. At the end of the experimental period the investigations revealed significant decrease in haemoglobin concentration, packed cell volume, total erythrocyte count and leucocytosis with heterophilia leading to clinical anaemia.

INTRODUCTION

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. There are many groups of pesticides which are commonly used in agriculture. These include Algicides, Bactericides, Insecticides, Fungicides, Acaricides, Molluscicides, Nematicides, Rodenticides and Virucides. Among these, fungicides and insecticides are commonly used in agriculture. Fungicides are chemical compounds or biological organisms used to kill or inhibit fungi or fungal spores. The fungicides which are commonly used in orchards of Jammu and Kashmir state include Capton, Dithionon, Mancozeb, Zineb and Ziram. Ziram is used widely on fruit and nut trees, apples, vegetables and tobacco. The indiscriminate use of fungicides and pesticides on fruit crops has led to the decline of many birds and beneficial insect populations. Insects feeding on the sap of the apple tree or buds die instantly during the spraying of these pesticides. After eating the poisoned insects, the birds become drowsy and are not able to fly (Ajaz 2010). Ziram has not been studied extensively for its toxic effects on birds. Ziram has been proven more toxic than Ferbam and Thiram in adult fowl (Rasul & Howell 1974). Although there are benefits of the use of pesticides, but on the other hand toxicity of pesticides to humans, birds and other animals pose a potential threat. Keeping in view the paucity of information regarding the toxicity of the chemical, this study was undertaken to observe haematological changes in broiler chickens.

MATERIALS AND METHODS

A total number of 150 healthy broiler chicks were procured from the market and used for experimental purpose. All the chicks were vaccinated against Marek's disease on the first day of hatching. The chicks were given Furasol @ ½ gram per litre of water for 5 days in the first week of the experiment used to prevent coccidiosis in chicks. The chicks were given broiler starter ration for two weeks, followed by broiler finisher ration as per standards.

The chicks were reared up to 2 weeks of age. After that the chicks were divided into four groups viz. acute, subacute, subchronic and control on random basis. The acute group consisted of 10 chicks, subacute 20 chicks, subchronic 90 chicks and control 30 chicks. The control group received fungicide free diet, whereas acute, subacute and subchronic groups received fungicide at different dose rates given below

Induction of toxicity in chicks: For inducing toxicities, the product as available in the market was used. Ziram was available 80% in the market. The dose rate was selected on the basis of LD50 by dividing the number of days in each toxicity group. The LD50 of Ziram in birds is 100mg/kg body weight (Michael, 1997). For inducing acute toxicity single

oral dose of Ziram @100mg/kg body weight (LD50 dose) was given to the experimental birds. For inducing subacute toxicity, LD 50 dose was given orally in divided doses daily for 20 days i.e. 5mg/kg body weight per day. For inducing subchronic toxicity, LD 50 dose was given orally in divided doses daily for 90 days i.e. 1mg/kg body weight per day. The experimental design is as follows.

		Acute Group	Sub acute Group	Sub chronic Group	Control Group
1	Dose	100mg/kg b.wt.	5 mg/kg b.wt.	1 mg/kg b.wt.	Nil
2	Route	oral	oral	oral	NIL
3	No. of birds	10 birds	20 birds	90 birds	30 birds
4	Day of blood collection	24 hours interval	10th and 20th day	10 days interval	10 days interval
5	Sacrifice of birds	24 hours interval	10th and 20th day	10 days interval	Along with other group
6	No. of birds scarified at once	10 birds	10 birds	10 birds	3 birds

Haematological studies: The investigation was carried out manually or using MS4 haematological analyser with reagents from Erba chemicals. The parameters studied included: 1. Total erythrocyte count; 2. Total leukocyte count; 3. Differential leukocyte count; 4. Haemoglobin; 5. Packed cell volume.

Statistical analysis: Results are expressed as mean±S.E. with equal to number of animals. Data were analysed by t-test, one-way ANOVA followed by Dunnet's test and two-way ANOVA followed by Bonferroni's multiple comparison tests using SPSS software.

RESULTS AND DISCUSSION

Haemoglobin (g/dl): The mean haemoglobin concentration was significantly (P<0.05) decreased at 24 hours of study period when compared with corresponding values of control or the values at zero day shown in Table 1. In case of subacute group, the mean haemoglobin concentration of Ziram intoxicated birds did not vary significantly upto 10th day and were comparable with that of control and the values of zero day, but there was a significant decrease in the mean values of haemoglobin of intoxicated birds when compared with the values of control and then at zero day. The ziram intoxicated birds present in the subchronic group revealed a progressive decrease in the mean haemoglobin concentration till the end of the experiment and these values were significantly lower (P<0.05) than the values at zero day and also age matched control birds from the 10th day onwards.

Total erythrocyte count (M/mm³): The mean value of TEC shown in Table 2 in Ziram intoxicated birds at 24 hours interval was significantly (P<0.05) lower when compared with the values at zero day. However, the values did not differ significantly from that of corresponding values of control group at 0 day and 24 hours interval. The birds of subacute

Table 1: Effect of acute, sub-acute and sub-chronic ziram intoxication on haemoglobin concentration (g/dl) in broiler chickens (Mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	11.86±0.68^{Aa}	11.29±0.43^{Aa}
24 hours	11.00±0.97^{Aa}	7.36±0.27^{Bb}
Sub acute group		
Day 0	12.03±0.97^{Aa}	11.71±0.31^{Aa}
Day 10	12.13±0.68^{Aa}	12.09±0.34^{Aa}
Day 20	10.76±0.78^{Aa}	7.87±0.36^{Bb}
Sub chronic group		
Day 0	12.20±0.81^{ABa}	12.63±0.22^{Aa}
10 days	10.90±0.94^{BCa}	7.72±0.29^{BCb}
20 days	11.46±0.53^{AB}	7.86±0.38^{BCb}
30 days	10.76±0.84^{BCa}	8.01±0.37^{Bb}
40 days	11.70±0.72^{ABa}	7.40±0.28^{BC}
50 days	11.70±0.52^{ABa}	7.16±0.30^{BCb}
60 days	10.70±0.87^{BCa}	7.22±0.29^{BCb}
70 days	11.53±0.66^{ABa}	6.97±0.26^{BCb}
80 days	12.7±0.20^{Aa}	6.71±0.35^{BCb}
90 days	9.50±0.529^{Ca}	6.37±0.27^{Cb}

Means in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

Table 2: Effect of acute, sub-acute and sub-chronic ziram intoxication on total erythrocyte count (M/mm³) in broiler chickens (Mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	2.99±0.14^{Aa}	3.21±0.07^{Aa}
24 hours	3.00±0.21^{Aa}	2.72±0.10^{Ab}
Sub acute group		
Day 0	3.01±0.20^{Aa}	3.18±0.08^{Aa}
Day 10	3.07±0.18^{Aa}	3.13±0.06^{Aa}
Day 20	3.04±0.19^{Aa}	2.64±0.09^{Bb}
Sub chronic group		
Day 0	3.03±0.11^{Aa}	3.05±0.10^{Aa}
10 days	3.10±0.13^{Aa}	2.64±0.09^{ABa}
20 days	2.90±0.16^{Aa}	2.65±0.08^{ABa}
30 days	3.03±0.26^{Aa}	2.69±0.07^{ABa}
40 days	2.83±0.12^{Aa}	2.55±0.13^{Ba}
50 days	2.98±0.21^{Aa}	2.48±0.08^{Bb}
60 days	2.91±0.16^{Aa}	2.54±0.07^{Ba}
70 days	2.91±0.23^{Aa}	2.44±0.10^{Ba}
80 days	2.81±0.21^{Aa}	2.48±0.05^{Ba}
90 days	2.94±0.13^{Aa}	2.51±0.11^{Ba}

Means in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

Table 3: Effect of acute, sub-acute and sub-chronic ziram intoxication on packed cell volume (%) in broiler chickens (Mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	26.60±2.05 ^{Aa}	27.13±1.31 ^{Aa}
24 hours	26.33±1.82 ^{Aa}	21.92±0.88 ^{Bb}
Sub acute group		
Day 0	27.16±0.54 ^{Aa}	29.69±1.18 ^{Aa}
Day 10	26.86±1.00 ^{Aa}	30.72±1.16 ^{Aa}
Day 20	26.20±1.00 ^{Aa}	24.82±1.00 ^{Ba}
Subchronic group		
Day 0	26.80±0.63 ^{Aa}	31.29±0.89 ^{Ab}
10 days	26.63±1.09 ^{Aa}	27.81±1.0 ^{Ba}
20 days	27.23±1.72 ^{Aa}	24.21±0.59 ^{Ba}
30 days	25.60±1.55 ^{Aa}	26.95±0.93 ^{Ba}
40 days	26.40±1.90 ^{Aa}	26.10±0.92 ^{Ba}
50 days	25.20±0.60 ^{Aa}	26.76±0.82 ^{Ba}
60 days	25.46±1.53 ^{Aa}	26.69±0.79 ^{Ba}
70 days	25.50±2.31 ^{Aa}	25.33±0.39 ^{Ba}
80 days	24.26±1.18 ^{Aa}	26.89±1.05 ^{Ba}
90 days	26.43±1.82 ^{Aa}	28.37±0.68 ^{Ba}

Means in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

Table 4: Effect of acute, sub-acute and sub-chronic ziram intoxication on total leukocyte count (Th/mm^3) in broiler chickens (Mean±SE)

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	24.30±2.57 ^{Aa}	23.54±0.91 ^{Aa}
24 hours	23.83±1.92 ^{Aa}	33.93±1.65 ^{Bb}
Sub acute group		
Day 0	25.20±2.58 ^{Aa}	26.96±0.99 ^{Aa}
Day 10	24.70±1.80 ^{Aa}	24.81±0.71 ^{Aa}
Day 20	25.26±2.40 ^{Aa}	33.00±0.86 ^{Bb}
Sub chronic group		
Day 0	24.76±1.60 ^{ABa}	24.36±0.80 ^{Aa}
10 days	25.00±0.92 ^{Aa}	31.20±1.26 ^{Bb}
20 days	19.33±2.54 ^{Aa}	32.82±1.42 ^{Bb}
30 days	24.40±2.51 ^{Aa}	31.60±1.49 ^{Bb}
40 days	24.90±2.40 ^{Aa}	31.88±1.40 ^{Bb}
50 days	24.76±2.23 ^{Aa}	33.58±1.26 ^{Bb}
60 days	21.63±2.89 ^{Aa}	33.04±1.30 ^{Bb}
70 days	24.60±2.17 ^{Aa}	32.32±1.33 ^{Bb}
80 days	19.90±2.65 ^{Aa}	33.60±1.43 ^{Bb}
90 days	23.66±1.35 ^{Aa}	32.51±1.07 ^{Bb}

Means in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

group revealed a progressive decrease in the values from 0 day to 20th day in the intoxicated group. However, values varied significantly at 20th day when compared with the values at 0 day, 10th day and the values of TEC in the control group at 20th day. In case of subchronic group, a progressive decrease in the values of TEC was noted in intoxicated birds with the mean counts significantly (P<0.05) lower than those at day 0 from day 40 of Ziram post intoxication. However, a non-significant decrease in TEC was also observed in control birds and also the mean values of the two groups did not differ significantly throughout the study period.

Table 5A: Effect of experimental acute Ziram intoxication on mean differential leukocyte count (%) in broiler chickens.

DLC	Days	Control	Intoxicated
Lymphocyte (%)	Day 0	39.10±1.28 ^{Aa}	36.81±0.63 ^{Aa}
	24 hr	32.80±1.45 ^{Aa}	25.20±3.24 ^{Ab}
Monocyte (%)	Day 0	8.033±0.58 ^{Aa}	7.74±0.43 ^{Aa}
	24 hr	10.63±1.00 ^{Aa}	9.60±1.09 ^{Aa}
Heterophil (%)	Day 0	48.50±1.51 ^{Aa}	49.99±0.94 ^{Aa}
	24 hr	50.20±0.50 ^{Aa}	71.25±4.13 ^{Bb}
Eosinophil (%)	Day 0	4.00±1.15 ^{Aa}	4.10±0.50 ^{Aa}
	24 hr	5.76±0.82 ^{Aa}	3.57±0.96 ^{Aa}
Basophil (%)	Day 0	0.36±0.12 ^{Aa}	0.360±0.06 ^{Aa}
	24 hr	0.60±0.11 ^{Aa}	0.380±0.07 ^{Aa}

Means for a parameter in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

Packed cell volume (%): The mean PCV value of intoxicated birds at 24 hours of Ziram intoxication was significantly (P≥0.05) lower when compared with values at 0 day or corresponding values of the control as presented in Table 3. A significant (P<0.05) decrease in the mean values of PCV of subacute group was observed in Ziram intoxicated birds at day 20, when compared with either values at 0 and 10th day mean values of control birds at different days. The mean values of PCV in case of subchronic intoxicated birds showed a progressive decrease from day 10 onwards. However, the mean values of PCV of intoxicated and control groups did not differ significantly at any point of time during the study period.

Total leukocyte count (M/mm^3): A significant (P≥0.05) increase was observed in the mean of TLC value at 24 hours interval in acute intoxicated birds when compared with the values of either control or values at 0 day. In case of subacute birds, no significant alteration in mean TLC values was observed at day 10 of the study. However, at day 20 post-intoxication, the mean values of TLC in intoxicated birds were significantly (P≥0.05) higher when compared with the values at 0 day or the corresponding values of the control group. A reference to the Table 4 revealed that the mean values of TLC in subchronic post intoxicated birds was increased significantly (P≥0.05) from day 10 and onwards when compared with baseline mean values or with those values of age matched control birds.

Differential leukocyte count (%): The mean values of DLC of acute group revealed significant (P<0.05) increase in the

Table 5B: Effect of experimental sub-acute Ziram intoxication on mean differential leukocyte count (%) in broiler chickens.

DLC	Group	Day 0	Day 10	Day 20
Lymphocyte (%)	Control	37.43±0.70 ^{Aa}	31.26±0.66 ^{Aa}	30.90±0.65 ^{Ba}
	Intoxicated	36.43±0.63 ^{Aa}	35.33±0.59 ^{Aa}	21.51±1.42 ^{Bb}
Monocyte (%)	Control	7.66±0.86 ^{Aa}	8.06±0.97 ^{Aa}	8.26±0.28 ^{Aa}
	Intoxicated	7.64±0.46 ^{Aa}	7.74±0.46 ^{Aa}	7.12±0.44 ^{Bb}
Heterophil (%)	Control	51.16±2.27 ^{Aa}	51.96±2.31 ^{Aa}	49.93±0.44 ^{Aa}
	Intoxicated	51.57±0.96 ^{Aa}	52.47±0.99 ^{Aa}	67.54±1.61 ^{Bb}
Eosinophil (%)	Control	3.33±1.33 ^{Aa}	3.66±0.88 ^{Aa}	3.10±0.05 ^{Aa}
	Intoxicated	3.80±0.53 ^{Aa}	3.80±0.53 ^{Aa}	2.61±0.43 ^{Aa}
Basophil (%)	Control	0.40±0.10 ^{Aa}	0.26±0.08 ^{Aa}	0.46±0.06 ^{Aa}
	Intoxicated	0.33±0.05 ^{Aa}	0.33±0.05 ^{Aa}	0.62±0.04 ^{Aa}

Means for a parameter in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

Table 5C: Effect of experimental sub-chronic Ziram intoxication on mean differential leukocyte count (%) in broiler chickens

DLC	Group	Day 0	10 days	20 days	30 days	40 days	50 days	60 days	70 days	80 days	90 days
LYM (%)	Control	34.96 ^{Aa} ±1.17	36.26 ^{Aa} ±1.35	35.10 ^{Aa} ±1.48	35.56 ^{Aa} ±1.94	32.80 ^{Aa} ±1.23	33.30 ^{Aa} ±0.66	32.43 ^{Aa} ±1.45	32.83 ^{Aa} ±0.68	32.63 ^{Aa} ±1.13	34.06 ^{Aa} ±1.15
	Intoxicated	34.43 ^{Aa} ±0.62	29.22 ^{ABb} ±1.49	27.15 ^{Ba} ±2.93	20.37 ^{Cb} ±1.30	17.61 ^{CDb} ±1.27	15.33 ^{CDb} ±0.71	17.56 ^{CDb} ±1.33	17.74 ^{CDb} ±1.51	11.98 ^{Db} ±0.67	12.73 ^{Db} ±0.64
HET (%)	Control	52.63 ^{Aa} ±2.68	49.90 ^{Aa} ±0.26	50.06 ^{Aa} ±0.17	50.43 ^{Aa} ±0.12	50.83 ^{Aa} ±0.18	48.26 ^{Aa} ±0.44	51.06 ^{Aa} ±0.37	49.53 ^{Aa} ±0.40	49.96 ^{Aa} ±1.62	50.53 ^{Aa} ±0.65
	Intoxicated	53.37 ^{Aa} ±1.03	56.34 ^{Aa} ±1.61	68.17 ^{Bb} ±1.14	67.98 ^{Bb} ±1.54	67.61 ^{Bb} ±1.22	65.38 ^{Bb} ±1.60	69.00 ^{Bb} ±0.38	68.28 ^{Bb} ±0.50	58.65 ^{Ab} ±3.80	67.09 ^{Bb} ±1.55
MON (%)	Control	7.63 ^{Aa} ±1.23	7.96 ^{ABa} ±0.84	9.33 ^{ABa} ±1.07	8.93 ^{ABa} ±1.42	10.33 ^{Ba} ±0.69	11.90 ^{Ca} ±0.41	11.16 ^{Ca} ±0.97	11.40 ^{Ca} ±0.61	11.56 ^{Ca} ±0.89	9.70 ^{ABa} ±0.70
	Intoxicated	7.74 ^{ACa} ±0.46	11.32 ^{Bb} ±0.52	7.6 ^{ACa} ±0.28	7.46 ^{Aa} ±0.28	7.52 ^{Ab} ±0.29	8.85 ^{ABb} ±0.44	7.41 ^{Ab} ±0.37	7.20 ^{Ab} ±0.47	10.20 ^{BCa} ±1.03	8.51 ^{ACa} ±0.69
EOS (%)	Control	3.66 ^{Aa} ±0.88	5.40 ^{Aa} ±0.30	5.00 ^{Aa} ±0.63	4.70 ^{Aa} ±0.70	5.46 ^{Aa} ±0.54	6.03 ^{Aa} ±0.63	5.20 ^{Aa} ±0.49	5.73 ^{Aa} ±0.37	6.06 ^{Aa} ±0.06	5.50 ^{Aa} ±0.26
	Intoxicated	3.80 ^{Aa} ±0.53	2.41 ^{Aa} ±0.27	5.89 ^{ABa} ±1.48	8.72 ^{BCa} ±0.92	6.66 ^{Ba} ±1.33	7.34 ^{Ba} ±1.15	7.47 ^{Ba} ±0.50	6.64 ^{Ba} ±0.56	4.10 ^{Aa} ±0.87	3.39 ^{Aa} ±0.44
BA (%)	Control	0.33 ^{Aa} ±0.18	0.46 ^{Aa} ±0.08	0.50 ^{Aa} ±0.05	0.36 ^{Aa} ±0.12	0.56 ^{Aa} ±0.08	0.50 ^{Aa} ±0.05	0.46 ^{Aa} ±0.06	0.50 ^{Aa} ±0.05	0.43 ^{Aa} ±0.03	0.53 ^{Aa} ±0.06
	Intoxicated	0.33 ^{ACa} ±0.05	0.71 ^{Ba} ±0.05	0.67 ^{Ba} ±0.05	0.51 ^{BCa} ±0.05	0.65 ^{BDa} ±0.05	0.69 ^{Ba} ±0.05	0.14 ^{Ab} ±0.01	0.15 ^{Ab} ±0.01	0.40 ^{CDa} ±0.06	0.64 ^{Ba} ±0.05

Means for a parameter in different columns bearing at least one common 'uppercase alphabet' superscript, and in different rows bearing at least one common 'lowercase alphabet' superscript does not differ significantly.

relative percentage of heterophil and corresponding significant decrease in the relative percentage of lymphocytes when compared at 24 hours post intoxication as given in the Table 5. Evaluation of DLC of subacute group revealed a significant (P≤0.05) decrease in mean percentage of lymphocyte and monocyte counts and increase in mean percentage of heterophil count of the intoxicated birds when compared with the age matched controls. The mean eosinophil and basophil counts did not reveal any significant (P≥0.05) variation during the experimental period. In case of subchronic group, there was significant increase in heterophil count and relative decrease in lymphocyte counts as observed from day 20 onwards when compared with zero day or age matched control birds. As per reference of the Table 5, the monocyte count also showed significant (P≤0.05) increase than the values of control birds from day 20 to day 70 but the means were comparable to mean values of day 0. The mean eosinophil counts were significantly higher than the baseline means from day 30 to 70, but the means did not differ significantly from those of age matched control birds. The mean basophil counts were significantly (P≤0.05) increased from day 10 to 50 and again at days 80 and 90 when compared with day 0 mean, but the means did not differ significantly from those of control at any point of time during the study period.

Reports available in the literature regarding the effects of Ziram carbamate on haematological parameters were, reduced haemoglobin concentration, packed cell volume and

total erythrocyte count. The results of the present study indicated significant decrease in packed cell volume, haemoglobin concentration and total erythrocyte count in all the Ziram intoxicated birds leading to the development of anaemia. To ascertain the mechanism of development of anaemia, as observed in the present study, a detailed investigation would be needed into Ziram induced haemolysis, impaired haemoglobin synthesis or impaired erythropoiesis.

Carbamate induced lysis of sheep RBC leading to the development of the anaemia has been reported by Casale (1989). The iron chelating property of carbamates also supports the impaired haemoglobin synthesis or impaired erythropoiesis as reported by Kanchi (2013). Low PCV together with decline in Hb and TEC revealed liver damage accompanied with anaemia which has also been reported earlier by Yousef (2003) in ruminants due to Thiram carbamate toxicity. Vanleeuwen et al. (1984) reported a fall in haemoglobin content due to Thiram toxicity in the rainbow trout.

In the present study there was significant increase in total leukocyte count and differential leukocyte count in all the three Ziram intoxicated groups. Leukocytosis as a result of Thiram carbamate intoxication has also been reported by Sandhu & Barar (2009) in ruminants. Pathological leucocytosis has been observed in carbamate intoxication by Edwards et al. (1991). The occurrence of neutrophilic leukocytosis associated with lymphocytopaenia in Thiram toxicity in rabbits has been reported earlier by Dickhaus & Heisler (1980). Chesterman et al. (1998) reported neutrophilia in beagle dogs after exposure to Thiram in order to meet demand of the body to combat with infection.

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