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Hematological Studies on White Male Rats Exposed to Some Antimoulting Compounds

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For more than a decade, the pesticide industry has been conducting research work on the synthesis and use of insect growth regulators with widely varying mechanisms of action. It constitutes a big step in the direction of selective insecticides and also opens up new avenues for integrated pest control. (Chang 1978 ; Lacey and Mulla 1978 ; Schaefer et al. 1978). However, these compounds when synthesized must be toxicologically safe to the non-target species.

From the laboratory experiments that have been conducted with 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Dimilin) ; 2-chloro-N-(4-(trifluoromethoxy) phenyl amino carbonyl) benzamide (SIR) and N-(2,6-difluorobenzoyl)-N'-(3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy) phenyl) urea (IKI), it was concluded that these compounds when administered orally at sub-chronic doses to rats, rabbits and beagle dogs, no significant differences were noted between control and treated groups in mean body weight, growth rates or total food consumption. The only effects observed were marginal increases in methemoglobin levels (unpublished data).

This study will present the effect of these compounds on the hematological picture in male rats.

MATERIAL AND METHODS

Twenty male Swiss-albino rats, locally bred at the High Institute of Public Health, Alexandria University, were used in the present study. They were 3 months old and weighed about 90 gms. The animals were put on a diet composed of bread soaked in milk alternating with dry wheat grains. They were divided into 4 groups of five animals each. Group I was given 96.7 mg/Kg of Dimilin each for 48 days i.e. a total of 4640 mg/Kg of Dimilin in corn oil solution. Group II was given 104.2 mg/Kg

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of SIR each for 48 days i.e. a total of 5000 mg/Kg of SIR in corn oil solution. Group III was given 145.8 mg/Kg of IKI each for 48 days i.e. a total of 7000 mg/Kg of IKI in corn oil solution. For control purposes, group IV was given 0.2 ml of corn oil for 48 days. At the end of the treatment, animals were killed. Blood samples were taken for the following hematological studies:

1. Hemoglobin was determined by the alkaline hematin method as described by Clegg and King (1942).
2. Hematocrit (Packed cell volume) was estimated in percent by the microtechnique method. Whole blood is centrifuged at a constant g, and the total volume of the red cell mass is expressed as a percentage of the whole blood volume.
3. Total erythrocyte count was done (million/cumm).
4. Total and differential leucocytic count were done (10^3/cumm and percentage of 100 cells counted, respectively).
5. Absolute indices were calculated:
   a. Mean cell volume (MCV)
      This is the volume of the average red cell, expressed in cubic microns.
      $$\text{MCV (cu u)} = \frac{\text{hematocrit (as a percentage)}}{\text{red cell count (in millions)}} \times 10$$
   b. Mean cell hemoglobin (MCH)
      This is the weight of hemoglobin in the average red cell, expressed as micro micro grams of hemoglobin.
      $$\text{MCH (uugm)} = \frac{\text{hemoglobin (gm percent)}}{\text{red cell count (in millions)}} \times 10 \text{ uugm}$$
   c. Mean cell hemoglobin concentration (MCHC)
      This is the concentration of hemoglobin per unit volume of red cell, expressed as a percentage.
      $$\text{MCHC (\%)} = \frac{\text{hemoglobin (gm percent)}}{\text{hematocrit (as a percentage)}} \times 100\%$$

RESULTS AND DISCUSSION

The mean hemoglobin concentration (gm/100 ml blood) of the Dimilin and SIR treated groups was significantly lower than that of the control group.

The mean hematocrit percent of the Dimilin and IKI treated groups was found to be significantly higher than that of the control group.

The mean erythrocyte count (million/cumm) of the SIR treated group was significantly lower than that of the control group.

The mean leucocyte count (10^3/cumm) of the SIR and IKI treated groups was significantly lower than that of the control group (Table 1).

Table 1. Hematological findings in rats exposed to Dimilin, SIR and IKI.

	Control	Dimilin	SIR	IKI
Hemoglobin (gm/100 ml)	17.50 ± 0.71	13.50* ± 0.71	14.00* ± 0.35	15.87 ± 0.88
Hematocrit (%)	39.33 ± 1.15	45.00* ± 0.00	38.00 ± 2.83	45.67* ± 5.13
Erythrocyte count (million/cumm)	7.34 ± 0.29	7.41 ± 1.04	6.33* ± 0.42	7.36 ± 0.74
Leucocyte count (10^3/cumm)	10.23 ± 1.44	9.20 ± 0.85	6.48* ± 1.93	7.95* ± 1.78

* Significantly different from control ($P < 0.05$).

Table 2. Differential leucocyte count in rats exposed to Dimilin, SIR and IKI.

	Control	Dimilin	SIR	IKI
Neutrophil	37.50 ± 3.53	32.00* ± 1.73	29.50* ± 2.12	36.00 ± 9.54
Eosinophil	1.50 ± 0.71	4.33 ± 2.31	2.67 ± 1.53	3.00 ± 1.73
Basophil	0.50 ± 0.70	2.00* ± 0.00	1.67 ± 0.58	0.67 ± 0.58
Lymphocyte	54.50 ± 0.71	57.67 ± 4.72	58.33 ± 7.37	58.00 ± 5.20
Monocyte	3.00 ± 1.41	4.00 ± 2.00	4.67 ± 0.58	3.50 ± 2.12

* Significantly different from control ($P < 0.05$).

Table 2 summarizes the differential leucocyte count in rats exposed to Dimilin, SIR and IKI. A decrease in the neutrophil count was noticed which was significant in the Dimilin and SIR treated groups. The basophil count showed an increase which was significant in the Dimilin

treated group. Also, an increase was noticed in the eosinophil, lymphocyte and monocyte counts.

Table 3 indicates the absolute blood indices in rats exposed to Dimilin, SIR and IKI. An increase in the MCV value was detected which was significant in the IKI treated group. MCH value in the Dimilin treated group was significantly lower than that of the control group. Also, MCHC value in the three tested groups was significantly lower than that of the control group.

Table 3. Blood indices in rats exposed to Dimilin, SIR and IKI.

	MCV (cu u)	MCH (u ug)	MCHC (%)
Control	53.17 ± 4.02	23.13 ± 1.85	44.87 ± 0.18
Dimilin	61.28 ± 8.59	17.83* ± 2.72	30.00* ± 1.57
SIR	62.38 ± 6.37	24.98 ± 3.52	36.97* ± 3.68
IKI	69.83* ± 0.28	22.76 ± 3.60	36.41* ± 1.55

* Significantly different from control ($P < 0.05$).

The results of this study showed that the hematological picture in the male rats treated with these antimoulting compounds was disturbed. There was a significant erythrocytopaenia in the SIR treated group. This effect may be due to the toxic effect of this compound on bone marrow as it has been reported by French and Macfarlance (1970) that some toxic drugs and chemicals affecting bone marrow may cause erythrocytopaenia. Gupta and Paul (1977) reported a significant reduction in total RBC count after ingestion of Malathion sprayed-fodder in buffalo calves. The leucocyte system serves to defend the body against foreign organisms or extraneous materials. The results revealed that a damage occurred to the defense mechanism of the rats treated with these compounds which was followed by eosinophilia, basophilia and monocytosis. The lymphocyte count was increased which means that there is a production of antibody carried out by lymphocytes to overcome the extraneous materials. Also,

a decrease in the neutrophil count was observed. The response to a fall in circulating neutrophils is an increased outpouring from the blood or storage in the bone marrow or destruction of the neutrophils themselves. Junqueira and Carneiro (1983) reported that under the action of certain toxic substances such as streptolysin (Streptococcus toxin), the neutrophils undergo rupture of the membranes of the granules, resulting in a tumefaction process followed by agglutination of the organelles and destruction of the neutrophils themselves. Moreover, an elevation in the temperature of the rats during the treatment was observed. This is probably due to the neutrophils responsible for endogenous pyrogen production and, therefore, fever (Smith, 1975).

The results showed significant reduction in the hemoglobin concentration and in the mean cell hemoglobin concentration (MCHC). These reductions lead to the development of hypochromic type of anemia. It should be pointed out that the term hypochromic refers to the hemoglobin concentration of red cells (Wintrobe, 1962). The prevalence of hypochromia points to the major contribution of iron deficiency. MCHC is a sensitive measure for the diagnosis of iron deficiency and indicates that hypochromia may point to block in iron utilization, infection or reduced hemoglobin synthesis.

On the basis of the present study, it seems reasonable to assume that these antimoulting compounds produced toxic effects on hematologic function. They developed macrocytic hypochromic type of anaemia with some reduction in RBC and WBC counts. Thus, care should be taken and more studies will increase the validity of this information.

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