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HAEMOGRAM AND HAEMATOPIOSIS OF MALE ALBINO RATS AS TREATED WITH DINICONAZOLE FUNGICIDE [59]

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ABSTRACT

The prersent investigation is concerned with the assessment of detrimental effect of diniconazole fungicide on haemogram in male ablino rats. The results indicate that the erythrocytes, leucocytes, platelets and reticulocytes counts, as well as haemoglobin content, packed cell volume (PCV), the mean corpuscular volume (MCV) and the myeloid: erythroid ratio in bone marrow did not change significantly of rats treated with diniconazole fungicide. The Heinz bodies did not detect in erythrocytes of treated rats. The mean corpuscular haemoglobin and the mean corpuscular haemoglobin concentration (MCHC) were reduced markedly with 25 and 50 ppm of diniconazole on the 45th day. The values returned to the normal level during the recovery period. Diniconazole fungicide did not induce any significant differences in neutrophils and lymphocytes counts, while the eosinophils count was significantly decreased at 12 ppm on the 90th day from treatment. The osmotic fragility of erythrolytes did not change during the experimental period.

Key words: Haemogram, Haemotopiosis, Rats, Fungicide, diniconazole

INTRODUCTION

The major source of environmental contamination by pesticides is the deposits resulting from their application to control agriculture as well as public health pests. Diniconazole is a new triazole fungicide and shows excellent control of powdery mildew on grape, apple and peach, as well as of rust disease on apple.

Roe *et al* (1979) reported that a single spray of monocrotophos insecticide (Nuvacron 40) did not affect significantly the count of erythrocytes (RBCs) and leucocytes count (WBCs) and haematocrite values in volunteers.

The oral administration of isoproturon herbicide (at low doses) to male albino rats revealed no significant changes in packed cell volume (PCV), haemoglobin (Hb) values, WBCs and RBCs as well as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values while at higher

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doses there was significant increase in WBCs and lymphocytopenia and neutrophilia. These scientists also observed no change in neutrophil, lymphocytes, monocytes and eosinophils counts at lower doses of tested compounds (**Sorkar and Gupta, 1993**).

Use of phoxim (OP) at concentrations (2000 ppm) on rams induced a significant increase of erythrocytes count, haemoglobin and haematocrite values (**Nasr, *et al.*, 1996**).

The present investigation aimed to throw light on the influence of diniconozole fungicide on the haemogram and haematopiosis of treated albino rats in laboratory.

MATERIAL AND METHODS

1. Source and maintenance of animals

Male albino rats were purchased from Organization of Biological Serum Products and Vaccine and maintained in the laboratory on balanced diet, (**El-Maassarawy, 1996**).. The animals were allocated randomly into four main groups. One as the control group and the other three groups for diniconazole treatments.

2. Fungicide used

A formulation sample of diniconazole 5% EC [E-(1)-2,4 dichloro-phenyl) 4-4-dimethyl-2-(1,2,4 triazole-1-yl) 1-penten-3-ol] was used in drinking water and provided for the animals *ad-libitum*. The active ingredient content in the drinking water was adjusted to 12, 25 and 50 ppm. Each animal group was allocated randomly into six subgroups. Five subgroups represented the treatment periods, (15, 30, 45, 60 and 90 days). and were kept for 30 days (recovery period) after treatment. The 6th subgroup received plain drinking water as control group.

3. Blood sampling and haemogram assessments

At the end of each period, the blood samples were collected by orbital sinus technique, in small labelled dry and clean epeindorff tubes containing EDTA [Ethylene DiamineTetraacetic acid] as anticoagulant (1 mg/1 ml fresh blood] (Schalm, 1986). The erythrocytes count (RBCs), leucocytes count (WBCs), Packed cell volume (PCV), haemoglobin concentration (Hb) and differential leucocyte count (**Schalm, 1986**) were measured.

4. Bone marrow aspiration and cells differentiation

Bone marrow was aspirated from the femur of treated rats with syringe needle and 2 ml of fetal calve serum. The bone marrow cells were centrifugated at 3000 r.p.m for 5 minutes. The supernatent were discarded, the cells were suspended and made bone marrow smear. In each bone marrow smears, the cells were differentiated into erythroid, myeloid; and the unidentified cells were excluded. The ratio between myeloid and erythroid cells was calculated.

5. Reticulocytes and Heinz bodies detection

Counting reticulocytes and detection of Heinz bodies were carried out on the blood films prepared after staining with Brilliant cresyl Blue. (**Schalm, 1986**).

6. Blood platelets count

Counting of blood platelets was done by using haemocytometer chamber and special diluent (1% Ammonium Oxalate) (**Schalm, 1986**).

7. Erythrocytes Osmotic Fragility (EOF)

The resistance of erythrocytes to haemolysis was measured by subjecting it, to decreasing concentrations of NaCl solutions (**Parpart, 1947**).

8. Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by **Snedecor and Cochran (1980)**. Treatments means were compared by the least significant test (L.S.D) at the 5% level of probability. Also, statistical analysis of some data was carried out according to the method of **Dixon and Massay (1957)**.

RESULTS

Data in Table (1) demonstrate a significant decrease in erythrocyte count after 30 days with diniconazole treatment at 12 and 25 ppm. During the recovery period the erythrocyte values reached normal levels. Diniconazole exhibited significant reduction in haemoglobin content after 45 days from administration of the three tested concentrations used. The prolongation of time indicate recovery occurrence and haemoglobin content returned to the normal levels (Table 1).

The Packed cell volume (PCV) showed different response to diniconazole concentrations. The lowest concentration caused significant decrease in packed cell volume after 60 days of treatment. The medium concentration (25 ppm) caused increase in PCV after 15 and 45 days (by 46% and 39%); while PCV decreased after 60 days (36.6%). The highest concentration (50 ppm) caused significant reduction in PCV after 30 days (34%). Diniconazole did not change the platelets count in treated rats within the experimental period.

Data in Table (2) indicate that the different concentrations of diniconazole did not induce, in general, significant differences in mean corpuscular volume (MCV) in treated animals compared with normal level. The highest concentration (50 ppm) induced significant increase in this parameter after 45 days from treatment and the values returned to normal level during the recovery period. The same trend was observed with corpuscular haemoglobin concentration (MCH) except with diniconazole at 25 and 50 ppm after 45 and 90 days. Data also indicate that the three tested concentrations caused marked reduction in mean corpuscular haemoglobin concentration (MCHC) after 45 days from treatment. All treated animals recovered and reached to the normal level during the recovery period.

Data in Table (3) indicate that diniconazole did not alter the leucocytes count in treated rats during the experimental Course. Inadditiony diniconazole treatments had no significant effect on reticulocytes count throughout the experimental period. Moreover, examination of blood film showed no incidence of Heinz bodies in erythrocytes of diniconazole treated rats. Again, diniconazole at different concentrations did not impact on neutrophils and lymphocytes counts. The same trend was found with eosinophils count, except at 12 ppm concentration

Table 1. Effect of treatment with different concentrations of diniconazole on erythrocytes count, haemoglobin conc., packed cell volume, platelets count and reticulocytes counts of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
	Erythrocytes count (x 10^6/µl)						
Control (0.0 ppm)	6.238	7.100	6.342	7.318	5.656	6.068	6.454
12 ppm	7.824	6.900	3.784*	6.608	6.684	6.622	6.404
25 ppm	7.090	7.312	5.516	7.124	7.382	7.384	6.977
50 ppm	7.488	7.300	3.194*	6.780	7.518	6.404	6.457
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 1.611							
	Haemoglobin concentration (g/dL)						
Control (0.0 ppm)	9.172	13.620	14.992	13.558	11.334	13.030	12.618
12 ppm	8.224	13.088	13.306*	12.636	12.234	12.160	12.108
25 ppm	9.338	14.210	12.516*	11.422	12.378	12.378	12.042
50 ppm	9.598	13.038	13.006*	12.400	11.98	11.524	11.938
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 1.530							
	Packed cell volume (%)						
Control (0.0 ppm)	43.00	37.000	35.000	41.000	34.400	38.800	37.800
12 ppm	43.00	37.200	35.800	36.600*	33.200	36.200	37.00
25 ppm	43.200*	36.000	39.200*	36.600*	35.400	36.000	37.733
50 ppm	46.00	34.000*	33.600	39.000	37.000	34.800	36.667
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 2.940							
	Platelets count (x 10^9/µL)						
Control (0.0 ppm)	0.894	1.177	1.090	1.151	1.120	0.999	1.072
12 ppm	0.890	1.001	1.032	0.993	0.994	0.737	0.941
25 ppm	0.861	1.216	0.779	0.916	0.054	1.000	0.961
50 ppm	0.708	1.158	0.784	0.989	1.173	0.999	0.969
No significant differences exist between treatments No significant of interaction between period of treatment x treatment concentration							

Table 2. Effect of treatment with different concentrations of diniconazole on mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin conc. of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Mean corpuscular volume (MCV) (FI)							
Control (0.0 ppm)	69.238	49.214	58.558	62.650	58.836	56.884	59.230
12 ppm	55.112	55.254	63.108	60.070	49.786	54.562	56.315
25 ppm	60.826	49.398	62.892	51.636	47.670	54.550	54.495
50 ppm	61.078	45.510	39.038*	59.186	49.912	52.714	60.240
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 15.119							
Mean corpuscular haemoglobin (MCH) (Pg)							
Control (0.0 ppm)	13.588	17.588	24.124	18.876	19.092	21.762	19.014
12 ppm	18.250	18.250	22.290	18.600	19.242	19.250	18.088
25 ppm	19.942	18.942	19.000*	16.992	15.436	23.564	17.917
50 ppm	17.276	17.276	20.362*	17.852	16.020	17.684	17.237
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 3.256							
Mean corpuscular haemoglobin conc. (MCHC((%)							
Control (0.0 ppm)	22.496	39.618	41.716	34.188	34.986	3.676	34.945
12 ppm	19.166	33.152	38.152*	32.526	36.384	34.450	32.366
25 ppm	22.176	37.510	31.606*	33.876	33.196	35.434	32.800
50 ppm	22.494	38.200	37.820*	31.780	30.926	32.916	32.356
L.S.D. of treatment means = 1.7611 L.S.D. of interaction between period of treatment x treatment concentration = 4.314							
Reticulocytes (%)							
Control (0.0 ppm)	0	0	0	0	0	0	0
12 ppm	0	0.5	0	0	0	0	0
25 ppm	1.5	0	0	0	0	0	0.5
50 ppm	1.0	0	0.5	1.5	1.5	1.5	0.5
No significant differences exist between treatments. Non significant of interaction between period of treatment x treatment concentration.							

Table 3. Effect of treatment with different concentrations of diniconazole on leucocytes, neutrophil, eosenophil and lymphocytes counts of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Leucocytes counts (x 10^3 µl)							
Control (0.0 ppm)	4.902	9.464	5.298	7.78	7.364	8.768	7.262
12 ppm	5.230	9.050	7.918	6.970	7.450	7.432	7.342
25 ppm	4.782	9.166	7.715	8.230	7.750	7.628	7.628
50 ppm	5.504	9.264	4.980	6.370	8.330	6.047	6.749
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Neutrophil count							
Control (0.0 ppm)	870.80	669.44	603.20	1187.53	764.60	1051.09	857.78
12 ppm	582.30	451.20	991.06	997.50	395.50	1034.80	742.06
25 ppm	885.12	587.71	858.46	1221.16	679.50	612.60	807.43
50 ppm	756.14	1087.64	973.81	803.30	953.76	940.96	919.27
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Eosenophil count							
Control (0.0 ppm)	319.380	79.332	207.74	132.10	317.60	403.76	264.98
12 ppm	224.02	114.60	320.52	212.40	220.00*	500.00	265.26
25 ppm	171.06	215.00	120.70	311.55	287.30	182.60	214.70
50 ppm	123.32	89.47	110.75	292.90	338.60	338.60	229.42
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 251.403							
Lymphocytes count							
Control (0.0 ppm)	4301.00	9443.50	5080.60	6599.87	6513.84	6372.13	6385.29
12 ppm	4084.40	8474.20	6588.12	5747.80	6707.60	6664.12	6377.71
25 ppm	3840.80	6244.70	6182.38	7590.52	8419.10	6933.00	6535.09
50 ppm	4467.72	9534.02	6862.32	5416.30	7309.76	5447.43	6506.26
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							

which showed significant decrease on the 90th day of treatment. Data indicate that diniconazole did not induce significant differences in myeloid and erythroid rates, as well as, myeloid : erythroid ratio between treated and untreated groups of animals.

It is clear from these observations that diniconazole concentrations did not affect the osmotic fragility of red blood cells in a uniform fashion with different treatments.

DISCUSSION

The blood has direct or indirect effects on the biochemical processes in living organisms, where it carries the necessities of life to all cells, of the body and it carries the wastes products of metabolism to the organs of excretion. Thereafter, it is possible to expect any alteration in the cellular content of blood when exposed to environmental pollutants.

Haematological investigation on rats treated with diniconazole revealed insignificant changes in erythrocytes count, haemoglobin content and packed cell volume throughout the experimental period. Such effects continued after withdrawal of the fungicide from the drinking water. A decrease in haematological concerns occurred on the 45th day.

The erythrocyte indices revealed no significant alteration in MCV and MCH during the experimental period and after withdrawal of the tested compound. The MCHC values showed significant decrease on the 45th day and returned to normal values after withdrawal of diniconazole.

These findings are in agreement with those of by **Roe *et al* (1979); Schlick and Friedberg (1981), Rinner *et al* (1981) and Sorkar and Gupta (1993)**, who mentioned that red blood cells, haemoglobin content, packed cell volume and erythrocytes indices, (MCV and MCH) did not alter after treatment of rats with different chemical substances such as monocrotophos, carbaryl, pentachlorosobenzene and isoproturon.

Moreover, **Kalkowska and Szubartowska (1986)** reported that when quails administered 50 mg/kg Ekatin showed significant reduction in mean corpuscular haemoglobin concentration (MCHC) and led to the development of hypochromasia, this may be due to iron deficiency, reduced iron utilization or reduced haemoglobin synthesis **(Wintrobe,*et al* 1974).**

Platelets play an important role in haemostasis and coagulation processes in the body and their origin in the bone marrow. The platelets count in treated rats showed insignificant changes throughout the experimental period. These findings support with the result of **Hanely *et al* (1984)** who reported that in rats inhaling ethylene glycol monethyl ether (EGME), the platelets count did not change significantly.

The total and differential leucocyte count of treated rats, showed that diniconazole did not induce any significant differences. This agrees with that studied by **Rinner *et al* (1981)** on effects of pentachloronitrosebenzene in rats; **Schlick and Friedberg (1981)** on lead in mice; and **Roe *et al* (1979)** on monocrotophos in volunteers.

Insignificant changes occurred in reticulocytes count (%) in treated rats. The Heinz bodies were not incident in different treatments groups. **Hashimoto and Sakamoto (1982); Kalkowska and Szubarlioska (1986)** reported that the reticulocytosis and basophilic stippling

was prominent in rats treated with N, M, methylene-bis-acrylamide (MBA), as well as in quails treated with ekatin. **Rinner *et al* (1981)** detected the Heinz bodies in rats with highest dose of pentochloronitrosbenzene.

The effects of diniconazole on haematopioesis in the bone marrow proved that the tested compound did not alter the myeloid and erythroid cells values as well as the myeloid : erythroid ratio which is similar to the results obtained by **Zhang *et al* (1995)** who reported that in mice treated with 6-hydroxy-trans-tranl-2,4,hexadienal (10 mg/kg/day) no changes in bone marrow cellularity was observed.

The results of the present work revealed that diniconazole treatments did not affect the erythrocytic osmotic fragility. Similar results were previously reported by **Hashimoto and Sakamoto (1982)**, who found that the osmotic fragility of erythrocytes in rats did not change after treatment with N, N, methylen-bis-acrylamide (MBA). Also, **Saini *et al* (1996)** reported that the osmotic fragility of erythrocytes remained unaffected after single dose of 73 or 294 mg propyleneglycol/100 g b.w. after 7 days of treatment.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(٢) ، ٨٢٧ - ٨٣٦ ، ٢٠٠٣

صورة وتخليق خلايا الدم في ذكور الفئران البيضاء المعاملة بمبيد الدينيكونازول

[٥٩]

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في هذه الدراسة تم تقييم الاثر الضار للمبيد الفطري الدينيكونازول علي صور الدم في ذكور الفئران البيضاء ، حيث تشير النتائج الي عدم حدوث تغيرات معنوية بصورة عامة في عدد كريات الدم الحمراء والبيضاء وكذلك في الصفائح الدموية وايضا في عدد الخلايا الشبكية اثناء فترة التجربة ، يضاف الي ذلك عدم حدوث تأثيرات في محتويات كريات الدم الحمراء من الهيموجلوبين وكذلك في حجم الكريات المنضغطة اثناء التجربة ، وايضا لم يحدث تغيرات معنوية في نوع ونسبة خلايا نخاع العظام للحيوانات المعاملة بمركب الدينيكونازول وكذلك لم يلاحظ تكون اجسام هاينز في كريات الدم الحمراء في الفئران المعاملة اثناء فترة التجربة ، علاوة علي ذلك لم يحدث تغير معنوي لمتوسط حجم كرة الدم الحمراء بينما حدث نقص معنوي

لمتوسط وزن الهيموجلوبين لكرات الدم الحمراء في حالة التركيزات المتوسطة والعالية (٢٥ ، ٥٠ جزء في المليون) في اليوم الخامس والاربعين من المعاملة ، أما بالنسبة لمتوسط تركيز الهيموجلوبين لكوات الدم الحمراء فقد حدث نقص معنوي مع التركيزات الثلاثة المختبرة في اليوم الخامس والاربعين من المعاملة • وقد عادت قيم هذه المعايير الي المعدلات الطبيعية اثناء فترة الاستشفاء • لم يسبب الدينيكونازول أي اختلافات معنوية في عدد الخلايا المتعادلة والخلايا الليمفاوية بينما حدث نقص معنوي في عدد الخلايا الحامضية بعد ٩٠ يوم من المعاملة بالتركيز المنخفض (١٢ جزء في المليون) ثم عادت المستويات الي المعدلات الطبيعية خلال فترة الاستشفاء . المعاملة بالدينيكونازول لم تحدث تغير في الهشاشة الاسموزية لكريات الدم الحمراء مع التركيزات المختلفة المستخدمة اثناء التجربة .

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