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Effect of Dimethoate on Some Haematological Parameters of Toad *Bufo regularis*

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Abstract. Males and females of Egyptian toads (*Bufo regularis*) were force fed dimethoate at a dose equivalent to the 0.5 LD_{50} (184 mg a.i./kg. b.w.). The control group was given saline solution only. The blood was collected from treated and control group in heparinized tubes at 24, 48, 72 and 96 hr. The following parameters were studied; the erythrocytes (RBCs) and leukocytes (WBCs) counts, haemoglobin (HGB) concentration, haematocrit value (HCT), mean cell volume (MCV), and mean cell haemoglobin concentration (MCHC).

In normal blood (control) the haematological parameters such as erythrocytes, leukocytes, haemoglobin, and haematocrit were higher in male than female. All the blood parameters were sharply decreased in the treated group in both males and females, except the mean cell volume, which was significantly increased. The shape of erythrocytes was changed during 96 hr. after treatment from oval to rounded to elongated, then to oval.

Introduction

Every day tons of synthetic chemical agents are being added into the human environment every where. These chemicals cause or contribute to the death, ill health or disablement of hundreds of millions [1, p. 282]. Insecticides are considered the most hazardous agents people are exposed to daily in the foood they eat, the water they drink and the air they breath. The changes in biochemical parameters as measured in various body fluids may often be among the more sensitive indicators of early changes in health due to hazardous exposure to insecticides in the environment [2].

The sublethal action of the organophosphate insecticide, dimethoate on animals has been studied for many years, and numerous effects have been described. They include increases in liver proteins and decreases in liver glycogen in rats [3, p. 421]; inhibition of spermatogenses in toads (*Bufo regularis*) [4]; increases of acid phosphatase in plasma and kidney, and alkaline phosphatase in liver and testis of male toad, *Bufo melanostictus* [5].

The present study was performed to investigate the effects of a single dose of dimethoate on some haematologicalparameters,which can be used as indicators of early changes in health as a result of exposure to dimethoate.

Materials and Methods

Sexually mature male and female Egyptian toads, *Bufo regularis,* weighing approximately 40 gm each, were collected by a regular supplier from Smouha district in Alexandria. The toads were kept in large glass aquaria with small amounts of water that was changed twice a day. The animals were fed twice a week on earth worms. They acclimatized ten days in the laboratory before testing. Animals were divided into two groups of 100 toads each (50 males and 50 females). The first group was subjected force-fed, each receiving 184.5 mg/kg body weight (0.5 LD_{50}) of dimethoate. The second group served as control in which each toad received 0.5 ml of amphibian saline solution (0.65% NaCl).

Stock solution of dimethoate was prepared and used for LD_{50} determination according to the method of American Public Health Association [6, p. 874].

Blood Collection

After specified regular time intervals (24, 48, 72 and 96 hr), five animals from each treatment were anesthetized. The hearts were exposed, and the pericardial sac was cut. The blood was withdrawn from a cardiac puncture into heparinized tubes.

Blood Counting

Erythrocytes (RBC's) and white blood cells (WBC's) were counted according to Yokoyama method [7, p. 120]. Haematocrite value (PCV) was determined adopting the method of Mukherjee [8, p. 150]. Haemoglobin concentration was measured by the method of Oser [9, p. 1472]. Absolute values, mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated. A drop of heparinized blood was transferred to a glass slide to make the smear which was subsequently stained with a commercial preparation of Giemsa-Ramanwsky. Eye-piece micrometer was used for red cell diameter measurement.

Statistical Analysis

The significant differences between two means were calculated with student's t-test. The differences considered significant and highly significant when the corresponding levels of probability were 0.05 and 0.01 respectively.

Results

1- Acute Toxicity of Dimethoate on *Bufo Regularis* Blood Parameters

The lethal doses that kill 50% of the tested animals (LD_{50}) were estimated after 24, 48, 72 and 96 hr of treatment. The LD_{50} values were found to be 509, 448, 415 and 369 mg a.i./kg body weight respectively.

The data in (Table 1) summerize the effect of a single dose equivalant to 1/2 LD_{50} of dimethoate (184.5 mg a.i./kg b.w.) on RBC's count, WBC's count, HGB concentration, HCT value, MCV and MCHC after 24, 48, 72 and 96 hr respectively. As shown in Table 1. RBC's were significantly decreased in treated groups of males and females compared to the control groups during the time intervals at which the test was

Table 1. Interaction of dimethoate (184.5 mg/kg, 0.5 LD_{50}) on some blood parameters of *Bufo regularis* at different time intervals after treatment.

Parameter	Sex		Elapsed time (hr)			
			24	48	72	96
	m	C	1.52±0.09	1.32±0.13	1.33±0.13	1.31±0.02
RBC's × 10^6	m	T	0.59±0.11**	0.51±0.07**	0.60±0.02**	1.04±0.16**
cell/mm	f	C	1.21±0.13	0.98±0.16	0.91±0.09	1.11±0.05
	f	T	0.34±0.02**	0.33±0.02**	0.50±0.05**	0.96±0.09**
	m	C	15.80±0.22	15.7±0.40	13.7±0.38	12.9±0.36
WBC's × 10^3	m	T	7.10±0.34**	6.9±0.27**	9.3±0.42**	10.9±0.58**
cell/mm	f	C	12.4 ±0.27	12.5±0.38	11.7±0.34	11.8±0.29
	f	T	6.4 ±0.43**	6.4±0.22**	8.2±0.40**	9.8±0.38**
	m	C	11.6±0.25	10.8±0.47	10.1±0.27	11.2±0.31
HGB	m	T	7.9±0.45**	6.9±0.42**	8.2±0.47**	9.6±0.25**
gm/dl	f	C	10.6±0.42	9.9±0.54	9.6±0.36	9.9±0.29
	f	T	6.9±0.42**	6.2±0.34**	6.7±0.34**	6.4±0.27**
	m	C	32.7±3.58	30.9±3.13	32.9±4.02	32.3±2.46
HCT (PCV)	m	T	25.8±4.92*	22.5±2.68**	25.3±3.58**	23.9±3.35**
%	f	C	30.2±4.25	29.1±2.91	29.6±3.35	31.9±1.79
	f	T	22.2±4.02*	20.3±1.79**	23.7±2.91**	21.1±2.91**
	m	C	21.5±2.91	23.4±3.80	24.7±2.01	24.5±2.68
MCV	m	T	43.7±3.35**	44.1±1.34**	42.2±2.01**	22.9±2.01
μ/RBC	f	C	25.6±3.13	29.7±3.80	32.5±3.35	28.7±2.46
	f	T	65.3±5.59**	61.5±2.91**	47.4±3.58**	22.0±4.25*
	m	C	35.5±2.68	35.0±1.57	30.7±1.34	34.7±2.01
MCH	m	T	30.6±2.01*	30.7±1.12**	32.4±2.01	40.2±2.46**
gm/dl	f	C	35.1±1.79	34.0±2.24	32.4±1.57	31.0±2.24
	f	T	31.1±2.46*	30.5±1.34*	28.3±1.79**	30.3±0.89

Each figure is the mean of 5 replicates ± SD

C = control group T = treated group

m = male f = female

** Highly significant at $P \leq 0.01$ * Significant at $P \leq 0.05$

performed. The total count of WBC's in the treated groups (males and females) were also significantly decreased. The count values showed that WBC's decreased greatly at the 1*st* and 2*nd* day, then started to increase at the 3*rd* and 4*th* day after treatment. All the other tested parameters were also significantly decreased in the treated groups, except mean cell volume in which significant increases were observed.

2- Effect of Dimethoate on The Blood Smears

The full mature formed erythrocytes of untreated toads were elliptical discoid elements with centrally located nucleus as visualized in fixed preparation (Fig. 1).

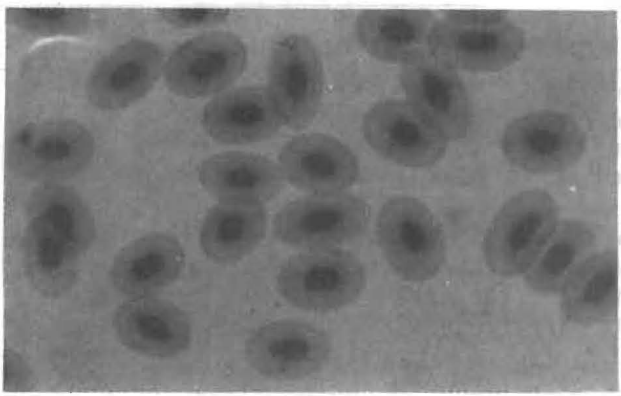

Fig. 1. Blood smear of *Bufo regularis* fed 0.5 ml amphibian saline (control) showing normal oval and nucleated erythrocytes.

In toads, treated with 0.5 LD_{50} of dimethoate, the shape was changed from oval to rounded to more oval, then to circular and oval again during four days of exposure. This denotes seme sort of recovery by lapse of time after treatment (Fig. 2). After 24 hr of treatment many abnormalities were observed such as the formation of buds (tongue like projections) on some erythrocytes, also a degree of hypochromia (hypochromic anaemia) have been observed as judged also by decrease in both erythrocyte number and haemoglobinig values. (Fig. 3). At 48 hr, the erythrocytes become more elliptical in the treated groups. The fixed erythrocytes of the control and the treated groups measured an average of 17.3 × 12 and 22.7 × 9.1 micron respectively. It was noticed that there were different shapes of the erythrocytes (pleiomorphism) during this time of treatment. The nuclei were densely stained at the periphery and a morphological change similar to that seen on the cell shapes was also observed on the nuclei shapes, (Fig. 4) and (Table 2). At 72 hr after treatment, the red blood cells of the treated groups, showed normal size (17.6 × 13.1 U), with circular and oval shapes, besides a degree of hypochromia, together with elongation and a mitotic division in the nuclei (Fig. 5). A mitotic division was also observed in the red cells after 96 hr of treatment (Fig. 6).

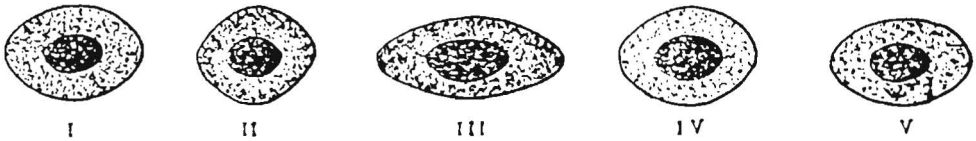

Fig. 2. Diagram showing the erythrocytes shape of *Bufo regularis* received a single dose of dimethoate (184.5 mg/kg).
I- Control erythrocyte.
II-V Erythrocyte after 24, 48, 72 & 96 hr of treatment.

N.B. Each one micron is represented by 0.2 mm.

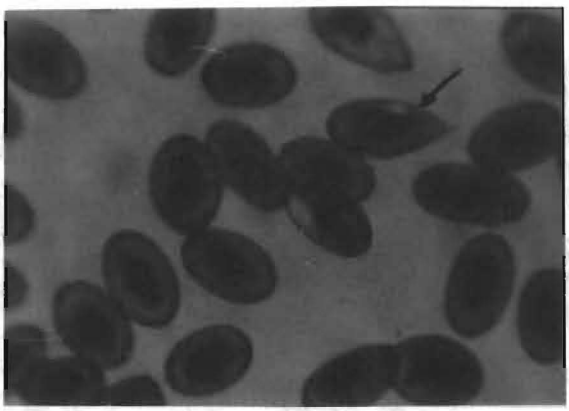

Fig. 3. Blood smear of *Bufo regularis* after 24 hr exposure to a single dose of dimethoate (184.5 mg/kg b.w.), showing erythrocytes with a degree of hypochromia and an erythrocyte with small protrusion.

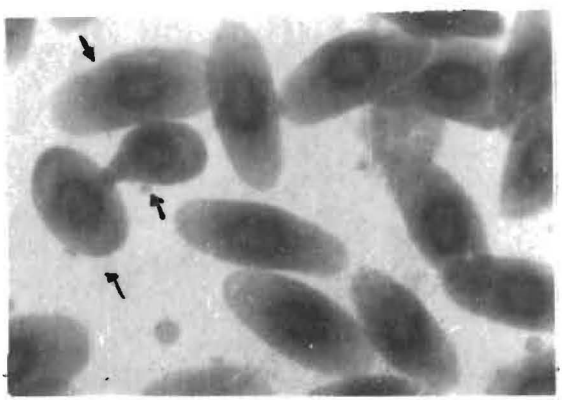

Fig. 4. Blood smear of *Bufo regularis* after 48 hr of exposure, showing more eleongated erythrocytes (poikilocytosis) and different shapes (pleiomorphism).

Table 2. Change of the erythrocytes diameters and their nuclei of *Bufo regularis* after feeding a single dose of dimethoate (184.5 mg/kg b.w.) for 96 hr.

The tested subject		cell diameter (micron) ± SD				
		Control group	Treated group			
			24 hr	48 hr	72 hr	96 hr
Red cell	width	17.3±0.5	14.6±1.5**	22.7±1.5**	17.6±0.5**	16.9±1.5**
	depth	12.0±2.0	12.0±0.5	9.1±0.5**	13.1±0.5**	11.8±1.5
Nucleus	width	6.8±1.0	6.3±0.5	7.6±1.5	6.7±0.5	6.3±1.0
	depth	4.8±1.0	4.3±1.5	5.7±1.5	5.4±1.0	4.3±0.5

** Highly significant at ($p \leq 0.01$)
each figure is the mean of 10 replicates.

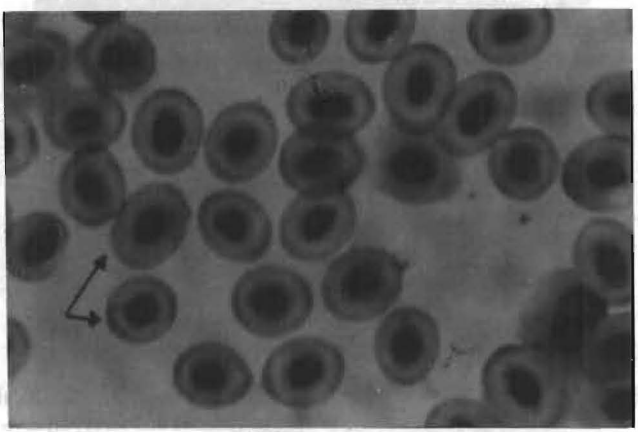

Fig. 5. Blood smear of *Bufo regularis* after 72 hr., showing different size of erythrocytes with severe hypochromia.

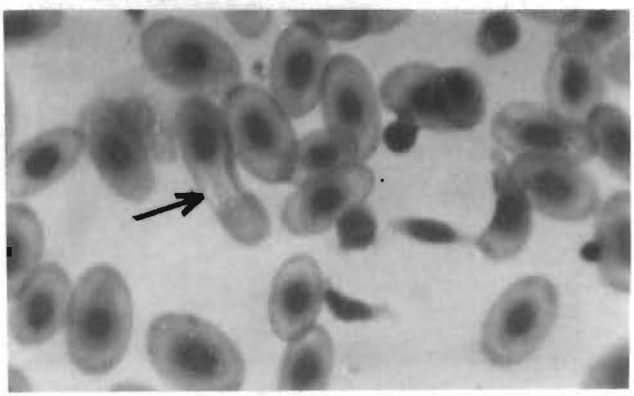

Fig. 6. Blood smear of *Bufo regularis* after 96 hr., showing a degree of hypochromia and an erythrocyte with budding.

Discussion

The data listed in this study expressed the *in vivo* interaction of dimethoate with different blood parameters. The data show significant decline in RBC's in the treated groups. This significant decrease suggests that dimethoate may cause great destruction of the blood corpusclus. This data agree with that obtained by Andrew [10, p. 188] and Shabana [11].

A significant decrease was also observed in the leukocytes count of the treated groups compared with the control groups. The data revealed that the haemoglobin concentrations, haematocrite values were significantly decreased as a result of dimethoate administration. It is noticed that haemoglobin concentrations and haematocrite values were directly correlated with RBC's count. This is due to the synergistic link among these blood parameters in all vertebrates. This relation was reported by Gatten and Brooks [12] on the tropical frog *Leptodactylus fallax,* and Hilmy *et al.* [13]. This close correlation between erythrocyte count, haemoglobin concentration and haematocrite value was also reported for other vertebrates including man [14].

The absolute values of MCV and MCHC have been widely used in the classification of anaemia. The increased MCV under such toxicant, mean primarily that there is macrocytic anaemia [15, p. 387]. The highly reproducible values for MCV and MCHC have become much more reliable aids to the recognition of minor degrees of macrocytosis or in the diagnosis of onset of iron deficiency at any early stage [16], and for discriminating between iron deficiency and thalassaemia trait [17]. The data recorded in this study revealed that MCV of the treated groups were significantly increased, which indicate the so called macrocytic anaemia. The MCHC in the treated groups diminished below the control groups, which indicate hypochromic anaemia (iron deficiency).

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تأثير المبيد الفوسفوري الدايمثويت على بعض الدلالات الدموية في الضفادع

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ملخص البحث. أجريت هذه الدراسة لمعرفة تأثير إعطاء جرعة واحد من مبيد الدايمثويت على مكونات الدم في الضفادع المصرية لمدة ٩٦ ساعة بعد المعاملة. قسمت الضفادع إلى مجموعتين كل مجموعة مائة ضفدع وأعطيت المجموعة الأولى جرعة المبيد عن طريق الفم، أما المجموعة الأخرى فأعطيت محلولاً ملحيًا فقط واستخدمت هذه المجموعة كمقارنة للمجموعة الأولى. وتم أخذ عينات الدم بعد ٢٤، ٤٨، ٧٢، ٩٦ ساعة من المعاملة. اتضح من النتائج أن كل مكونات الدم الآتية قد تأثرت بشدة نتيجةً للمعاملة بالمبيد: عدد كرات الدم الحمراء والبيضاء، تركيز الهيموجلوبين، قيمة الهيماتوكريت، متوسط حجم الخلايا، ومتوسط تركيز الهيموجلوبين في الخلايا، وانخفضت المكونات كلها انخفاضًا معنويًا، ماعدا متوسط حجم الخلايا الذي ارتفع معنويًا. أما شكل خلايا كرات الدم الحمراء فقد تغير من الشكل البيضاوي إلى الشكل المستدير ثم إلى الشكل المستطيل وأخيرًا إلى الشكل البيضاوي مرة أخرى، وصاحب ذلك ظهور نوع من الأنيميا الناتج عن قلة عد كرات الدم الحمراء مع ازدياد حجم هذه الخلايا.