

TOXIC EFFECT OF HERBICIDE "MACHETE" ON THE HAEMATOLGICAL PICTURE AND SPLEENIC TISSUE IN RATS

El-Mahrouky, Fatma S.

Plant Protection Research Institute, Agric. Res. Center, Dokki-Giza, Egypt

ABSTRACT

The effect of 1/4 LD₅₀ oral administration of herbicide "Machete" daily for 5 weeks on body weight and haematological parameters of albino rats including R.B.C.s, W.B.C.s, haemoglobin and packed cell volume were investigated. The herbicide caused a significant decrease in body weight and a slightly increase in W.B.C.s while red blood cells, haemoglobin and packed cells volume progressively insignificantly decreased. Histological changes of spleens were shown in the form of lymphoid depletion and hyperplasia in the white pulps, while the red one and sinusoids were hyperemic.

However, it was found that the use of sublethal doses of herbicides "Machete" has greatly affected the blood picture and spleen tissue of rats.

INTRODUCTION

The use of insecticides, herbicides, fungicides, nematicides, rodenticides and molluscicides had recently increased for agricultural and public health purposes. The application of these pesticides in soils will potentially lead to changes in the population of soil invertebrates either directly or indirectly (Edwards and Thompson, 1973). A wide spectrum of herbicides are actively applied in Egypt in order to control a wide variety of weeds living among several crops as rice, corn and peanuts and dry beans. Machete (butachlor) one of these herbicides has proved to be active in controlling such weeds which cause major problems to agriculture and water resources.

Accordingly, the side effects of herbicides toxicity on different animals were investigated. In Egypt, the average annual application of herbicides is rather high (El-Sebae, 1980). Many authors have dealt with acute and chronic toxicity of various herbicides (Flaherty *et al.*, 1992; Fournier *et al.*, 1992; Mikula *et al.*, 1992 and Roloff *et al.*, 1993).

Spleen tissues have received some attention by some investigators (Gibb *et al.*, 1988; Fournier *et al.*, 1992 and Wang *et al.*, 1993).

The objective of this study is to clarify the histological changes liable to occur on splenic tissue as well as some haematological parameters in rats treated with herbicide Machete.

MATERIALS AND METHODS

Herbicide used :

Machete is chemically known as N- butoxymethyl-2-chloro-2, 6-dimethylacetanilide, was obtained from Monsanto Co. LD₅₀ for rats 2000 mg/kg body weight.

Experimental animals :

Forty adult male rat with an average weight 100-120 gm were used in the present study. They were fed on a standard diet and water *ad libitum*. 5 rat served as control and the others were given oral daily dose of 1/4 LD₅₀ (200 mg/kg body weight) of herbicide Machete for 5 weeks. Animals from the control and treated were sacrificed after 1, 2, 3, 4 and 5 weeks post-treatment. The collected samples of rat blood from control and treated were processed for carrying out different haematological studies. Red blood cell count, white blood cell count, the blood cell volume, haemoglobin content were determined according to Schalm *et al.* (1975). Miller (1960), Wintrob (1934) and Levinson and Mac Fate (1956), respectively.

Pieces of examined spleen were fixed in Bouin's for histological study. They were then processed in paraffin wax for microtomy in sections of 5 µ thick and staining was carried out using haematoxylin and eosin method.

RESULTS

Table (1) revealed that the body weight of treated animals was significantly lower than control ones (133.8, 134.2, 136.8, 116.0 and 110.0 g at 1, 2, 3, 4 and 5 weeks post-treatment compared to control animals (149.4 g). Also, a significant increase occurred in the weight of spleen following Machete (herbicide) treatment at 1, 4 and 5 weeks post-treatment, while at 2 and 3 weeks there is a non-significant changes in spleen weight. Concerning the haematological pattern, data present in Table (2) revealed a slight decrease in red blood cell count of male albino rat giving 1/4 LD₅₀ of herbicide Machete where 8.33, 8.40, 8.55, 8.75 and 8.80 million/mm³ blood at 1, 2, 3, 4 and 5 weeks post- treatment, respectively. The same trend was observed in Hb content and P.C.V., no significant effect where 17.08 & 35.58, 17.10 & 35.62, 17.20 & 35.83, 17.25 & 35.93 and 17.32 & 36.06 when measured at 1, 2, 3, 4 and 5 weeks post treatment, respectively. At the same time, W.B.C.s count showed a significant increases allover the test periods where 9.53, 9.42, 9.60, 9.65 and 9.78 thousand/mm³ blood.

Table (1) : Effect of herbicide (Machete) on body and spleen weight of albino rat treated with 1/4 LD₅₀ daily for 5 weeks.

Parameter	Control (mean±S.E.)	Weeks post-treatment (mean ± S.E.)				
		One	Two	Three	Four	Five
Body weight (g)	149.4 ±1.6	133.8 ±1.9**	134.2 ±1.8**	136.8 ±3.0*	116.0 ±2.1**	110.0 ±2.3**
Spleen (g)	0.26 ±0.03	0.42 ±0.01**	0.25 ±0.01	0.24 ±0.01	0.44 ±0.01**	0.45 ±0.01**

Data are expressed as mean ± S.E. of 5 rats

* Significant at P > 0.05

** Significant at P > 0.01

Table (2) : Changes in blood picture of rat treated with 1/4 LD₅₀ of herbicide "Machete".

Parameter	Control	Weeks post-treatment (mean ± S.E.)				
	(mean±S.E.)	One	Two	Three	Four	Five
R.B.C.s million/mm ³ blood	9.40 ±0.07	8.33 ±0.3	8.40 ±0.34	8.55 ±0.3	8.75 ±0.34	8.80 ±0.35
W.B.C.s thousand/ mm ³ blood	7.58 ±0.20	9.53 ±0.11*	9.42 ±0.06*	9.60 ±0.12*	9.65 ±0.11*	9.78 ±0.08*
Hb (gm/100 ml blood)	18.20 ±0.05	17.08 ±0.37	17.10 ±0.40	17.20 ±0.44	17.25 ±0.46	17.32 ±0.08
Ht (%)	113.7 ±0.05	106.75 ±0.3	106.87 ±0.3	107.5 ±0.4	107.8 ±0.4	108.2 ±0.5
P.C.V. (%)	37.90 ±0.38	35.58 ±0.3	35.62 ±0.3	35.83 ±0.4	35.93 ±0.4	36.06 ±0.5

Data are expressed as mean ± S.E. of 5 rats

* Significant at P > 0.05

Histopathological changes in spleen tissue after one week post-treatment with 1/4 LD₅₀ herbicide (Machete) were shown in Fig. (2) in the form of hyperemic red pulps and sinusoids with lymphoid depletion in the white pulps, while after two weeks post-treatment (Fig. 3) revealed that the white pulps showed lymphoid hyperplasia. In addition, Fig. (4) at 3 weeks post-treatment hyperemia was observed on the red pulps and sinusoids while lymphoid hyperplasia was located in the white pulps. the same observation was seen in Figs. (5 & 6) at 4 and 5 weeks post-treatment with 1/4 LD₅₀ of herbicide (Machete), the white pulps showed lymphoid hyperplasia.

DISCUSSION

It was clear from the present results that the losses in body weight of rats after administration of herbicide Machete may be due to the loss of appetite. The same observation was noticed by Fouad and El-Saify (1990) when treated pigeon and chicken with Methomyl and Trichlorofon insecticides. The observed decrease in red blood counts of rat after daily oral administration of 1/4 LD₅₀ herbicide Machete may be attributed either to the depressive action of herbicide Machete on the haemopoietic system or to direct destructive effect on red blood cells. Also, the decrease in red blood cells count recorded in the present study is similar to the results reported by Rung *et al.* (1988) and El-Mahrouky (1984) and Mekkawy *et al.* (1996) they studied the effects of 3 mg/L and 6 mg/L doses of the herbicide Atrazine on some haematological parameter of *Oreochromis niloticus* and *Chrysichthys auratus*, they found a significant decrease (P < 0.01) in red blood cell count, haemoglobin concentration, haematocrite percentage, MCV, MCH and MCHC of both species.

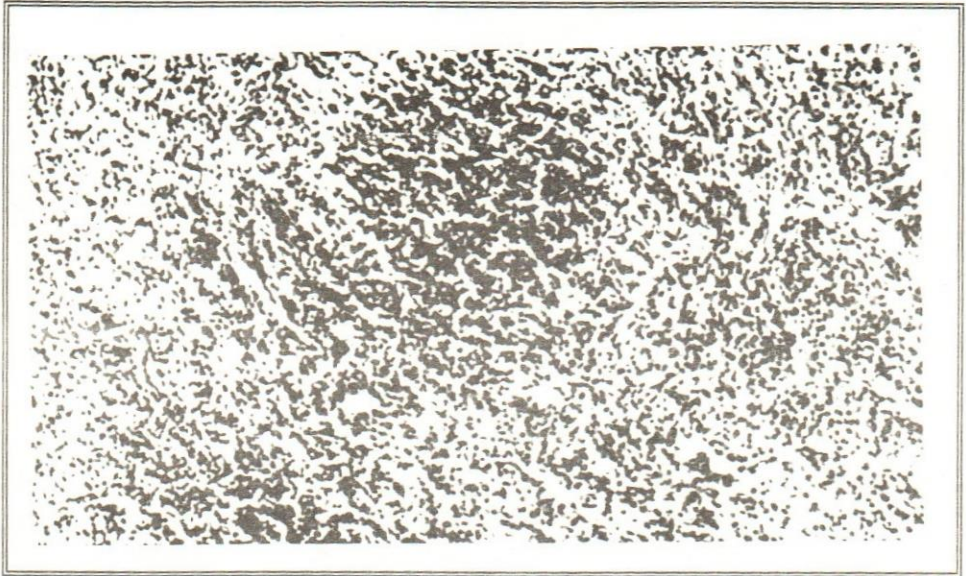


Fig. 1.- Spleen of rat in control group.

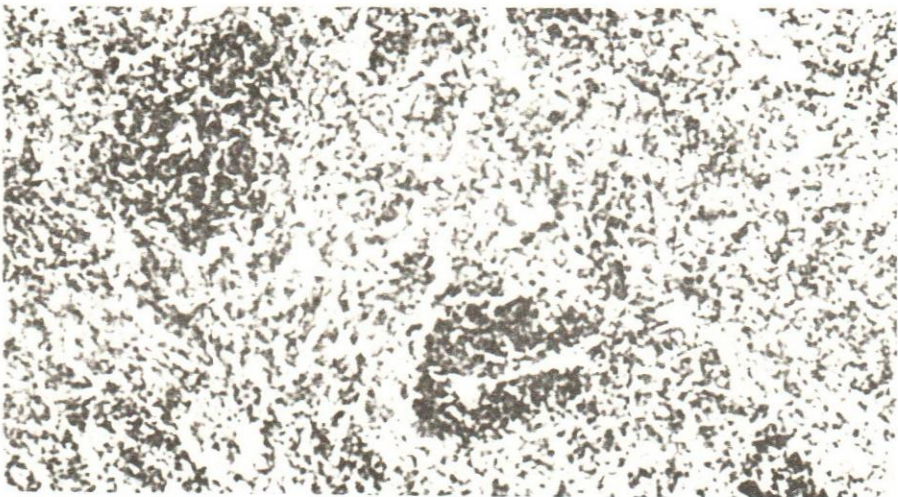


Fig. 2.- Spleen of rat treated and sacrificed after one week, showing hyperemic red pulps and sinusoids with lymphoid depletion in the white pulps (H & E : X40)

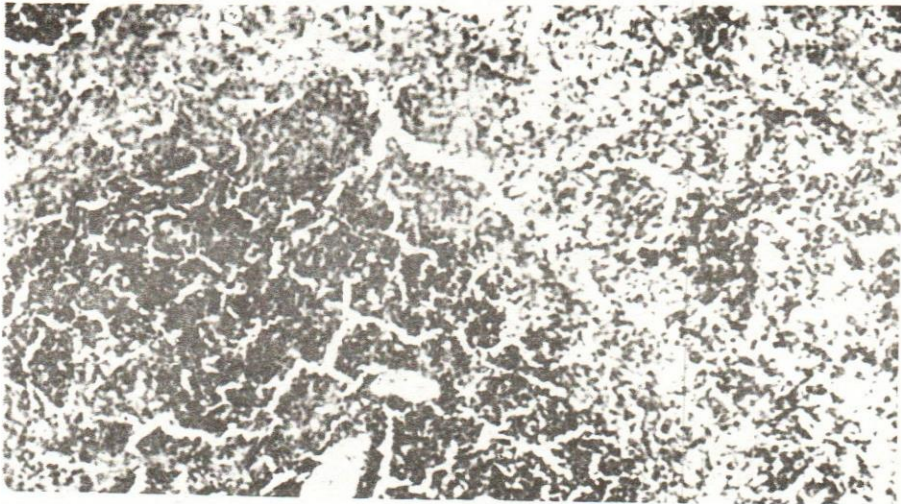


Fig. 3.- Spleen of rat treated and sacrificed after two weeks, showing lymphoid hyperplasia in the white pulps (H & E : X40)



Fig. 4.- Spleen of rat treated and sacrificed after three weeks, showing lymphoid hyperplasia in the white pulps with hyperemic red pulps and sinusoids

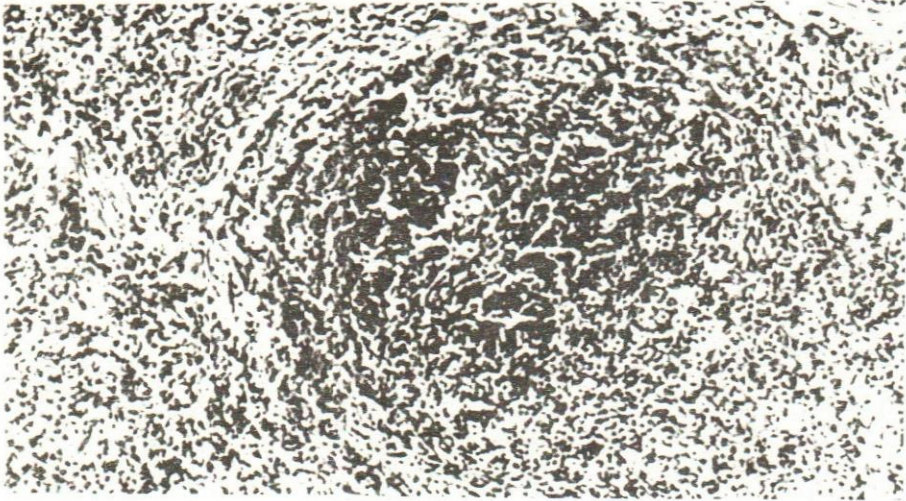


Fig. 5.- Spleen of rat treated and sacrificed after four weeks, showing lymphoid hyperplasia in the white pulps (H & E : X40)

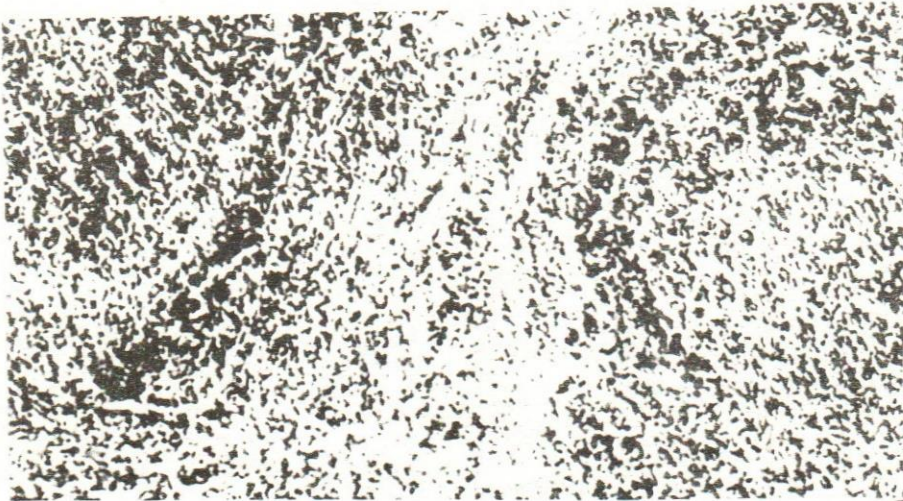


Fig. 6.- Spleen of rat treated and sacrificed after five weeks, showing lymphoid hyperplasia in the white pulps (H & E : X40)

Also, Prasad *et al.* (1991) referred to significant changes in some haematological constituents such as RBCs, Hb, PCV, MCV, MCH and MCHC of atrazine exposed *Tilapia mossambica*. They explained atrazine effect by the respiratory stress in the treated fish. Rehwooldt (1978) indicate that atrazine (herbicide) has a harmful effect on spleen, liver and anterior kidney.

The administration of 1/4 LD₅₀ of herbicide Machete caused a non-continuous increase of white blood cells. On the other hand, Nagata *et al.* (1992) studying another herbicide namely Paraquat, they found that an increase in segmented neutrophils and monocytes and decrease in lymphocytes. In the present study, the decrease in haemoglobin concentration and packed cell volume was noticed following the intake of herbicide Machete. The red cell production is dependent on the red cell mass and subsequently on haemoglobin concentration (oxygen carrying capacity). Any decrease in oxygen carrying capacity would subsequently lead to a decrease in red cell production. The present results are in accordance with those observed by Prasad *et al.* (1991) in fishes treated with atrazine herbicides. In the present study, histological lesions were observed in the form of necrotic degeneration, the white pulps showed lymphoid depletion and hyperplasia, while the red one and sinusoids were hyperemic. Similar results were reported by Gibb *et al.* (1988), who found necrosis tissue and central cavitation in spleen tissues of rat and mouse following MPTP (1-methyl-4 phenyl 1, 2, 3, 6 tetrahydropyridine) and MPP (1-methyl-4 phenyl pyridinium) intake. Fournier *et al.* (1992) recorded changes in spleen cell number and cell viability of mice treated with atrazine herbicides. Similar results were recorded by Wang *et al.* (1993), in the form of increased pigmentation and presence of haemosiderin after phenylurea herbicides intake in rats. Abdel-Hakim *et al.* (1995) revealed that the effect of T₄ on lymphocyte subpopulation in amphibian's spleen was not as marked in thymus. The total number of viable splenic cells fell slightly at day 7 after treatment with 50 µg T₄/g body weight. During 14 days post-treatment, there was a significant increase in the number of viable cells and eventually returned to baseline by 21 days.

Amer (1995) revealed that histological changes of spleens were shown in the form of thickening of capsules and trabeculae, fatty degeneration, atrophied follicles, necrosis and thickening of arteriolar walls.

Obviously, herbicides should be carefully used and haematological parameters should be frequently assessed.

REFERENCES

- Abdel-Hakim, S.; B. Wegdan; A.M. Hala and A.W.S. Mohamed (1995). Hormones and Amphibian immunity. VI- Effects of thyroxine on the lymphoid organs of *Bufo regularis*. J. Egypt. Ger. Soc. Zool., Vol. 18 (C) Histology & Histochemistry, pp. 63-91.
- Amer, Afnan M. (1995). Toxic effect of Bentazon on the haematological picture and splenic tissue in rabbits. J. Egypt. Ger. Soc. Zool., Vol. 18 (C) Histology and Histochemistry, pp. 233-249.
- Edwards, C.A. and A.R. Thompson (1973). Pesticides and the soil fauna. Residue Rev., 45 : 1-79.

- El-Mahrouky, F.S.M. (1984). Physiological studies on the response of rodents to some rodenticides. M.Sc. Thesis, Fac. of Science, Menoufia Univ., Egypt.
- El-Sebae, A.H. (1980). Biochemical challenges in future toxicological research. *J. Environ. Sci. Health*, 15 (6) : 689-721.
- Flaherty, D.K.; P.A. Winzeburger and C.J. Gross (1992). The effect of Lasso herbicide on human immune function as measured by *in vitro* assays. *Immuno Pharmacol Immunotoxicol.*, 14 (4) : 955-979.
- Fouad, S. and A. El-Saify (1990). Effect of Methomyl and Trichlorofon on body weight and some hematological parameters in two Egyptian birds. *J. Egypt. Ger. Soc. Zool.*, 1 : 1-13.
- Fournier, M.; J. Friborg; D. Girard; S. Mansour and K. Krzystyniak (1992). Limited immunotoxic potential of technical formulation of the herbicide atrazine in mice. *Toxicol. Lett.*, 60 (3) : 263-274.
- Gibb, W.R.; B. Gostali; A.M. Domency; M.E. Melly and R.J. Naylor (1988). The histological effects of intercerebral injection infusion of (1-methyl-4-phenylpndinium) and (1-methyl-phenyl 1, 2, 3, 6 tetrahydrop-ridine) in rat and mouse. *Brain Res.*, 4- 461 (2) : 361-366.
- Levinson, S.A. and R.P. Mac Fate (1956). *Clinical laboratory diagnosis*. 5th ed., 124 pp., Philadelphia, Lan and Febiger.
- Mekkawy, I.A.A.; S.Y. Hussein; M. Abdel-Nasser and Sh.M. Ahmed (1996). Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. *J. Egypt. Ger. Soc. Zool.*, Vol.19 (A) : 283-319.
- Mikula, I.; J. Pist and P. Kacmar (1992). The immune resonance of sheep to subclinical chronic exposure to the herbicide Bentazon-TP. *Vet. Hum. Toxicol.*, 34 (6) : 507-509.
- Miller, S.E. (1960). *A text book of clinical Pathology*. 6th ed., The Williams and Wilkins, Baltimore, U.S.A.
- Nagata, T.; I. Kono; T. Masaoka and F. Akahori (1992). Acute toxicological studies on Paraquat. Pathological finding in beagle dogs following single subcutaneous injections. *Vet. Hum. Toxicol.*, 34 (2) : 105-112.
- Prasad, T.A.; T. Srinivas; G.M. Rafi and D.C. Reddy (1991). Effect *in vivo* of atrazine on haematology and O₂ consumption in fish *Tilapia mossambica*. *Biochem. Int.*, 23 (1) : 151-161.
- Rehwoldt, R.E. (1978). Investigations into the acute toxicity and some chronic effects of selected herbicides on several freshwater fishes. *Appl. Ecol. J.*, 4 : 316-327.
- Roloff, B.D.; D.A. Bellux and L.F. Meisner (1993). Cytogenetic studies of herbicide interaction *in vivo* using Atrazine and Linuron. *Arch. Environ. Contam. Toxicol.*, 22 (3) : 267-271.
- Rung-Morris, M.A.; S. Lacob and R.F. Novak (1988). Characterization of hydrazine stimulated proteolysis in human erythrocytes. *Toxicol. Appl. Pharmacol.*, 94 (3) : 414-426.
- Schalm, O.W.; M.C. Jaim and E.J. Carroll (1975). *Veterinary Haematology*. 3rd ed., Lan and Febiger, Philadelphia, U.S.A.

- Wang, S.W.; C.Y. Chu; I.D. Hsu and C.J. Wang (1993). Haemotoxic effect of phenylurea herbicides in rats : role of haemoglobin adduct formation in splenic toxicity. *Food Chem. Toxicol.*, 31 (4) : 285-295.
- Wintrobe, M.M. (1934). Variation in size and haemoglobin content of erythrocytes in blood of various vertebrates. *Folia Haematologia*, 51 : 32-49.

دراسة التأثير السام لمبيد الماشيت على بعض قياسات الدم والطحال في الفئران
فاطمة شوقي المحروقي
معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى - الجيزة، مصر.

أجرى هذا البحث لدراسة تأثير ٤/١ الجرعة نصف المميتة من مبيد "الماشيت" يوميا عن طريق الفم لمدة ٥ أسابيع على كل من وزن الجسم وقياسات الدم للفأر الأبيض والتي تشمل : كرات الدم الحمراء، كرات الدم البيضاء، الهيموجلوبين وحجم الخلايا المترسبة. وقد أظهر تأثير المبيد نقص معنوي في وزن الجسم وزيادة بسيطة في أعداد كرات الدم البيضاء. بينما كان هناك تناقص كبير في كل من كرات الدم الحمراء، الهيموجلوبين، حجم الخلايا المترسبة ولكنه تناقص غير معنوي.

كما ظهرت التغيرات الهستولوجية في الطحال على هيئة نقص في الخلايا الليمفاوية وزيادة في الحويصلات (البصيلات) البيضاء، بينما كانت الحمراء منها محتقنة، بينما أظهر استخدام جرعة تحت المميتة من مبيد "الماشيت" تأثيرا قويا على صورة الدم وعلى أنسجة الطحال في الفئران.