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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (mean±S.E.)</th>
<th>Weeks post-treatment (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One</td>
<td>Two</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>149.4±1.6</td>
<td>133.8±1.9**</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.26±0.03</td>
<td>0.42±0.01**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E. of 5 rats
* Significant at P > 0.05
** Significant at P > 0.01

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Table (2): Changes in blood picture of rat treated with 1/4 LD$_{50}$ of herbicide "Machete".

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

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 LD$_{50}$ 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 LD$_{50}$ 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-Safty (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 LD$_{50}$ herbicide Machete may be attributed either to the depressive action of herbicide Machete on the haemopoeitic 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 Chrysichthyes auratus, they found a significant decrease (P < 0.01) in red blood cell count, haemoglobin concentration, haematocrit percentage, MCV, MCH and MCHC of both species.

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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. Rehwoldt (1978) indicate that atrazine (herbicide) has a harmful effect on spleen, liver and anterior kidney.

The administration of 1/4 LD$_{50}$ 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 T$_4$ 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 T$_4$/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 showed be carefully used and haematological parameters should be frequently assessed.

REFERENCES


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

أجريت هذه الدراسة لدراسة تأثير 1/4 الجرعة نصف المميتة من مبيد "الماشيت" يومياً عن طريق الفم لمدة 5 أسابيع على كل من وزن الجسم وقياسات الدم للفرائ الأبيض والأسود. وتشمل: كرات الدم الحمراء، كرات الدم البيضاء، الليمفاويات وحجم الخلايا المشرقة. وقد أظهر تأثير المبيد نقص ملحوظ في وزن الجسم وزيادة بسيطة في عدد كرات الدم البيضاء. بينما كان هناك تناقص كبير في كل من كرات الدم الحمراء، الليمفاويات، حجم الخلايا المشرقة. ولكن تناقص غير معنوي.

كما ظهرت التغييرات الهيستولوجية في الطحال على هيئة نقص في الخلايا الليفاوية وزيادة في الحويصلات (الباصيات) البيضاء، بينما كانت الحمراء منها محتفظة، بينما أظهر استخدام جرعة تحت المميتة من مبيد "الماشيت" تأثيراً قوياً على صورة الدم وعلى أنسجة الطحال في الفئران.